METABOLIC EFFECTS OF HIGH-INTENSITY INTERVAL TRAINING AND ESSENTIAL AMINO ACID SUPPLEMENTATION

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ABSTRACT

Katie Hirsch: Metabolic effects of high-intensity interval training and essential amino acid supplementation
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High-intensity interval training (HIIT) promotes rapid mitochondrial adaptation leading to increased cardiorespiratory fitness (VO_2), metabolic rate (RMR), and fat oxidation, in addition to promoting fat loss and increases in lean mass (LM). Nutritional intake around exercise is also known to modulate metabolic responses during and after exercise, which is further influence by sex. Essential amino acids (EAA) may support positive body composition and metabolic changes associated with HIIT, especially related to LM, but studies evaluating potential synergistic effects are lacking. The purpose of this study was to compare independent and combined effects of HIIT and EAA on body composition, muscle characteristics, and total body metabolism in overweight and obese adults; an exploratory aim was to evaluate the modulatory effects of sex. Sixty-six adults (50% female; Age: 36.7±6.0 yrs; %BF: 36.0±7.8%) were randomly assigned to 8wks of: 1) HIIT (2 days/week); 2) EAA supplementation (3.6g twice daily); 3) HIIT+EAA; or 4) control. Body composition, RMR, substrate metabolism, VO_2, and muscle characteristics were measured at baseline, 4wks, and 8wks; whole-body protein turnover and cardiometabolic blood markers were measured at baseline and 8wks. Results showed no significant changes in body composition ($p>0.05$). HIIT and EAA separately promoted increases in RMR (HIIT: +78.40 kcal/d) and fat oxidation (HIIT: +13%; EAA: +10%). HIIT and HIIT+EAA significantly increased VO_2, with an average increase of +5.1 ml/kg/min and +4.1 ml/kg/min after 8wks of HIIT and HIIT+EAA, respectively. HIIT and HIIT+EAA increased thigh LM size and quality, as indicated by increases in thigh LM (+0.2 kg) and vastus lateralis cross sectional area (+2.6 cm^2), volume (+58.45 cm^3), and echo intensity (-6.75 a.u.); improvements appeared to be enhanced by EAA supplementation, via an

increase in whole-body protein turnover (+1.0 g/kgBM/d). There were no significant changes in cardiometabolic markers ($p>0.05$). There was no sex interaction, indicating similar benefits in men and women. In conclusion, 8wks of HIIT, with and without EAA, did not improve total body composition, but increased thigh LM size and quality, while also promoting positive improvements in RMR, fat oxidation, and VO_2 in overweight and obese men and women.

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CHAPTER I

INTRODUCTION

Caloric restriction and physical activity are commonly recommended to promote weight loss, reduce cardiometabolic disease risk, and improve health outcomes associated with obesity [1, 2]. Current recommendations for promoting weight loss include 150 – 300 minutes of moderate intensity physical activity or 75-150 minutes of vigorous activity per week, in combination with two or more days of strength training and some form of caloric restriction [1, 2]. However, these recommendations underemphasize the effects of exercise intensity and macronutrient composition in the weight loss process, specifically the effects on body composition.

High-protein diets have been shown to positively influence weight loss outcomes, eliciting greater fat loss, while maintaining lean mass (LM), especially when combined with caloric restriction or exercise [3-6]. Although current recommended dietary allowance for protein is 0.8 grams of protein per kg of body mass per day ($g \cdot kg^{-1} \cdot day^{-1}$), there is strong evidence suggesting that this may not be sufficient to maintain protein balance, particularly with exercise [7]. Protein intakes of 1.2 – 1.6 $g \cdot kg^{-1} \cdot d^{-1}$ have been shown to promote positive physiologic and metabolic responses in individuals with type 2 diabetes and metabolic syndrome, while intakes of 1.2 – 2.4 $g \cdot kg^{-1} \cdot d^{-1}$ are recommended to promote recovery and adaptation from endurance and strength training [8, 9]. In a study by Arciero et al. (2014), overweight and obese adults who added a supplemental dose of 20g of whey protein, three times per day to their habitual diet had significant changes in body composition, losing fat mass and abdominal fat over the course of 16-weeks [3]. When combined with a multimodal exercise program, individuals improved insulin sensitivity, lost significantly more body fat, and gained a greater percentage of LM [3]. Beneficial effects of a high-protein diet, are attributed to the higher intake of essential amino acids (EAA), which

promote increased energy expenditure, enhanced fat oxidation, stimulation of muscle protein synthesis, and increased satiety [4, 5, 8].

Exercise is recognized as an important component of weight and metabolic health management, yet more than half of individuals do not meet minimum requirements for physical activity [10]. High-intensity interval training (HIIT) can promote significant improvements in cardiorespiratory fitness and metabolic health, comparable to moderate continuous exercise, but in a significantly shorter amount of time and reduced overall exercise volume [11]. This makes HIIT a feasible option for a variety of clinical populations who have limited exercise capacities and could benefit from more efficient training strategies [12]. Prior research on HIIT training has largely focused on the rapid aerobic and metabolic adaptations, which are attributed primarily to increased mitochondrial biogenesis and oxidative capacity [12]. However, less is known about the effects of HIIT when combined with dietary control, on body composition, particularly lean mass. Previous studies from our lab have shown that HIIT alone can elicit decreases in fat mass in overweight and obese women [13], while also potentially promoting increases in lean mass (LM) and muscle size in as little as three weeks [14, 15]. Fat loss with HIIT may be associated with post-exercise increases in energy expenditure and enhanced fat oxidation related to increased mitochondrial capacity [16, 17], while increases in LM may be associated with upregulation of mTOR, promoting myofibrillar protein synthesis [18]. Simultaneous improvements in cardiorespiratory fitness and fat loss/muscle gain with HIIT, would have significant health benefits.

Preliminary research from our lab has demonstrated that consumption of protein prior to a HIIT session augments post-exercise energy expenditure and fat oxidation, compared to carbohydrate [16] suggesting a potential synergistic effect between protein intake and HIIT. Although HIIT leads to rapid improvements in cardiorespiratory fitness and mitochondrial oxidation, if conducted in a fasted state, or if EAA availability is inadequate during recovery, chronic HIIT training could promote a negative protein balance [19]. Protein intake prior to and/or following an exercise bout has been shown to promote a positive protein balance, which would support mitochondrial biogenesis and muscle protein synthesis [19], ultimately promoting metabolic and body composition changes. Whey protein is commonly

recommended for promoting metabolic and body composition changes due to its high EAA content and rapid absorption [19]. Recently, EAA products, providing only the proportion of amino acids necessary to promote muscular growth, have become accessible to the public, providing a more efficient method of EAA ingestion, while also using fewer ingredients/fillers. Finally, there are known differences in substrate metabolism between men and women at rest and during exercise, with women showing a greater preference for fat oxidation, while men are more efficient at glucose metabolism [20]. Since HIIT and high protein diets have been shown to improve lipid oxidation, HIIT combined with protein/EAA supplementation may create a favorable metabolic environment to support weight loss, especially in women.

PURPOSE:

To compare the independent effects of HIIT and EAA supplementation on body composition and metabolism, and evaluate whether the combination of HIIT and EAA supplementation provides additional benefit.

SPECIFIC AIMS

Specific Aim 1: To compare the independent and combined effects of HIIT and EAA supplementation on body composition, muscle characteristics, and muscle architecture in overweight and obese men and women over the course of eight weeks.

Hypothesis 1: It was hypothesized that HIIT+EAA would result in greater improvements in body composition, specifically leading to decreased FM, percent body fat, and visceral adipose tissue and increasing LM, than HIIT, EAA, or CON.

Hypothesis 2: It was hypothesized that HIIT+EAA would result in greater increase in muscle size, as shown by an increase in cross sectional area (mCSA) and volume (MV), and decrease echo intensity (EI), indicating an improvement in muscle quality, than HIIT, EAA, or CON.

Specific Aim 2: To compare the independent and combined effects of HIIT and EAA supplementation on whole body metabolism, specifically whole body protein turnover, metabolic rate, substrate metabolism, and metabolic profile in overweight and obese men and women over the course of eight weeks.

Hypothesis 3: It was hypothesized that HIIT+EAA would significantly increase nitrogen balance, resulting in a positive nitrogen balance, compared to HIIT, EAA, or CON.

Hypothesis 4: It was hypothesized that HIIT+EAA would result in greater increases in resting metabolic rate (RMR) and fat oxidation, as indicated by a decrease in respiratory exchange ratio (RER), than HIIT, EAA, or CON.

Hypothesis 5: It was hypothesized that HIIT+EAA would result in greater increases in fasting concentrations of circulating metabolomic markers of fat oxidation and mitochondrial oxidative capacity, than HIIT, EAA, or CON.

Exploratory Specific Aim 3: To evaluate the modulatory effects of sex on body composition and whole body metabolic responses to EAA supplementation, HIIT, and a combination of the two.

Hypothesis 6: It was hypothesized that men would have greater changes in body composition, muscle characteristics, and muscle architecture in response to HIIT+EAA than women. Specifically, men would show greater loss of body fat, and greater increases in LM, mCSA, and muscle quality (decreased EI), than women.

Hypothesis 7: It was hypothesized that women would show more favorable metabolic changes in response to HIIT+EAA, exhibiting greater fat oxidation, as determined by RER and metabolomics markers of fat oxidation.

Hypothesis 8: It was hypothesized men and women would have similar improvements in cardiorespiratory fitness in response to HIIT.

DELIMITATIONS

1. Men and women between the ages of 25 – 50 years.

2. Overweight or obese status: body mass index (BMI) of 28 – 40 $kg \cdot m^{-2}$ and %BF ≥ 25% for men, and BMI of 25 – 40 $kg \cdot m^{-2}$ and %BF ≥ 30% for women.
3. Healthy, non-smokers, who were apparently free from disease, reporting no current or history of cardiovascular disease, diabetes, metabolic, pulmonary, renal, hepatic, gastrointestinal, musculoskeletal, mental disorders or medical or surgical events, such as bariatric surgery, heart surgery, or any joint or musculoskeletal surgeries occurring within 6-months prior to enrollment that would have significantly influence study outcomes or prevent safe participation, such as uncontrolled hypertension, an abnormal electrocardiogram, inconsistently taking medications (i.e. blood pressure medication, anti-depressants, anti-anxiety, hormonal contraceptives), or taking medications that may influence study outcomes (i.e. metformin, insulin, statins).
4. Women: eumenorrheic, reported consistent menstruation for three months prior to enrollment and were not pregnant or planning on becoming pregnant.
5. Participating in less than 150 minutes per week of moderate exercise, less than 2 days per week of resistance training, and were not currently participating in HIIT or had not participated in HIIT within 12 weeks prior to enrollment.
6. Weight stable: maintained weight (±eight pounds) within the three months prior to enrollment.
7. Not currently consuming a high protein diet (≥1.6 $g \cdot kg^{-1} \cdot day^{-1}$ and/or ≥25% of calories from protein) determined from a protein intake survey.
8. Not currently consuming meal replacements or dietary supplements that could influence LM or metabolism (i.e. protein, creatine, beta-alanine, carnosine, taurine, or beta-hydroxy beta-methylbutyate) within eight weeks prior to enrollment.
9. No known known sensitivities to the EAA treatment.
10. Not participating in another clinical trial within four weeks prior to enrollment that would influence study outcomes.
11. Did not have severely impaired hearing or speech or inability to speak English.

LIMITATIONS

1. Metabolomics measures metabolic products, but does not directly measure enzymatic content of energy producing pathways or mitochondrial content.
2. Echo intensity from ultrasound serves as an indirect measure of muscle quality, but does not differentiate between intramuscular connective tissue and intramuscular fat, which would require analysis of muscle biopsy.
3. Measurement of whole-body protein turnover provides information on the protein balance of the body, indicating states of protein synthesis or breakdown, but does not differentiate between myofibrillar muscle protein synthesis, mitochondrial biogenesis, or metabolic signaling pathways.
4. Measurements at baseline, 4-weeks and 8-weeks informs on chronic metabolic adaptations, but does not directly inform the rate at which these adaptations may occur.
5. Results may not be translatable to populations exhibiting chronic disease states, such as diabetes, cardiovascular disease, or cancer.
6. Results may translate differently to older (>50 years) and younger (<25 years) populations.

ASSUMPTIONS

Theoretical

1. Subjects accurately reported health and exercise history information.
2. Subjects adhered to pre-testing guidelines.
3. Subjects provided accurate dietary intake information on nutrition logs.
4. Subjects adhered to supplementation and accurately report EAA intake.
5. Subjects maintained normal daily activity and nutritional habits throughout the intervention.

Statistical

1. The population from which the sample will be taken was normally distributed.
2. The treatment groups were randomly assigned.

3. The sample variability was equal.

DEFINITION OF TERMS

Overweight and obese – body mass index (BMI) of 28 – 40 $kg \cdot m^{-2}$ and percent body fat (%BF) ≥ 25% for men, and BMI of 25 – 40 $kg \cdot m^{-2}$ and %BF ≥ 30% for women [21].

Fat mass (FM) – all extractable lipids from adipose and other tissues in the body.

Percent body fat (%BF) – fat mass expressed as a percentage of total body mass.

Lean mass (LM) – all residual lipid-free chemicals and tissues including water, muscle, connective tissue, organs, bone, and essential fats.

Total body volume (BV) – estimate of body size using dual-energy x-ray absorptiometry.

Total body water (TBW) – a measure of the intracellular and extracellular fluid compartments of the body using bioelectrical impedance spectroscopy.

Total body bone mineral density (Mo) – a measure of the bone mineral content of the body estimated using dual energy x-ray absorptiometry.

Visceral adipose tissue (VAT) –intra-abdominal adipose tissue estimated using dual energy x-ray absorptiometry.

Muscle cross sectional area (mCSA) – measure of muscular size (cm^2); determined by tracing the outline of the muscle along the fascial border [22, 23].

Echo intensity (EI) – an indirect measure of muscle quality; a quantitative gray-scale analysis of muscle composition from an ultrasound image that reflects contractile versus non-contractile (i.e. connective tissue and intramuscular fat) tissues [24].

Physiological cross sectional area (PCSA) – measure of muscle size (cm^2), accounting for muscle architecture; determined as muscle volume (cm^3) divided by fiber length (cm) [25].

Pennation angle (PA) – angulation of muscle fascicles; defined as the angle between the deep aponeurosis and two separate fascicles [26].

Fascicle length (FL) –length of muscle fascicles; defined as the distance between the superficial and deep aponeuroses [26].

Muscle volume – measure of muscle size from cross section ultrasound scan [27].

Whole body protein turnover – the flux or rate at which protein-bound nitrogen moves toward protein synthesis or protein breakdown.

Resting metabolic rate (RMR) – energy expended while at rest in a supine position, but still awake, as measured using indirect calorimetry.

Respiratory exchange ratio (RER) – a measure of substrate utilization that uses a ratio of carbon dioxide expired to volume of oxygen consumed to estimate the contribution of fat and carbohydrate to energy production.

Metabolomics – targeted metabolic profiling that involves comprehensive analysis of known circulating metabolic intermediates that can be used to identify signatures of different metabolic states and provide insight into mechanisms of metabolic substrate selection and energy pathways [28].

Cardiometabolic markers – Fasting blood glucose (mmol/L), total cholesterol (mmol/L), high density lipoproteins (HDL) (mmol/L), and non-high density lipoproteins *(*nHDL*)* (mmol/L).

Cardiorespiratory fitness (VO_2peak) – peak volume of oxygen consumed during a graded maximal exercise test.

High-intensity interval training (HIIT) – alternating sets (6-10) of one minute of pedaling at a resistance that corresponds with 90% max wattage and one-minute recovery at a self-selected resistance or complete rest.

Essential amino acids (EAA) – amino acids that cannot be synthesized by the human body and are essential for muscle growth and repair; L-leucine, L-lysine HCl, L-valine, L-isoleucine, L-arginine, L-threonine, L-phenylalanine, L-methionine, L-histidine, and L-tryptophan

SIGNIFICANCE OF STUDY

Results of this study improve understanding of how HIIT promotes both fat loss and muscle gain, and how EAA supplementation influences these changes. Specifically, results of this study provide insights into pathways through which HIIT and EAA supplementation promote physiological and metabolic adaptions to promote improvements in body composition and metabolic health. The combination of total body protein turnover and metabolomics, in addition to a multi-compartment body composition model and measures of muscle characteristics and architecture, provides a unique platform to evaluate muscular and mitochondrial adaptations to HIIT and EAA supplementation. This study also investigates the influence of biological sex on these adaptations. EAA intake and HIIT require minimal lifestyle changes and time commitment, respectively. This combined approach may be a more effective and sustainable approach for improving overall metabolic health compared to more traditional diet and exercise strategies. Simultaneous improvements in body fat and muscle mass from a reduced time-commitment exercise program could have significant implications for improving health outcomes and maintaining quality muscle mass in a variety of populations, especially those who have limited exercise capacities.

CHAPTER II

REVIEW OF LITERATURE

INTRODUCTION

Weight loss and the reduction of body fat are important components of improving metabolic health and reducing disease risk. A weight loss of 5-10% can effectively improve health outcomes associated with type 2 diabetes, cardiovascular disease, and other cardiometabolic diseases in overweight and obese adults [29, 30]. In order to achieve weight loss, a majority of governing bodies recommend a combination of caloric restriction and daily moderate physical activity [1, 30]. However, these recommendations do not directly consider effects on body composition, underemphasizing the importance of maintaining metabolically active lean tissue while also reducing body fat.

Macronutrient intake and exercise intensity can have varying impacts on body composition and metabolic health. High-protein diets, compared to traditional high-carbohydrate diets, have been shown to promote greater fat loss, while reducing loss of lean mass (LM). These effects have been attributed to improvements in muscle protein synthesis, energy expenditure, fat oxidation, and hunger regulation [4, 8, 19]. When combined with exercise, high-protein diets promote even greater fat loss, while maintaining or even increasing LM [3, 5, 6].

Moderate intensity, aerobic exercise is commonly promoted for improving health and stimulating fat loss due to the significant improvements in cardiorespiratory fitness and fat oxidation, associated with improved mitochondrial oxidative capacity [31, 32]. However, aerobic exercise is not a strong stimulus for promoting LM in healthy individuals [31]. In contrast, resistance training is promoted for increasing LM and strength due to its effects on muscle hypertrophy, but is less effective for promoting fat loss or improvements in mitochondrial adaptions [31, 32]. High-intensity interval training (HIIT) has gained

scientific and clinical traction due to the rapid improvements in cardiorespiratory fitness and mitochondrial oxidative capacity that can be achieved in significantly less time and volume than moderate intensity aerobic training [12, 33]. Recent evidence also suggests that HIIT promotes improvements in body composition, promoting fat loss and increases in LM [13-15, 18, 34, 35]. The potential for simultaneous improvements in cardiometabolic health and body composition from as little as two weeks makes HIIT an appealing exercise option, especially for clinical populations, who are not able to participate in a high volume exercise program. This review will evaluate mechanisms through which HIIT promotes improvements in body composition and metabolic health, particularly in regards to LM. This review will also evaluate how protein intake may support body composition and metabolic changes in response to HIIT, as well as potential modulatory effects of sex.

HIGH-INTENSITY INTERVAL TRAINING

Interval training is defined as short, vigorous bouts of physical activity, interspersed by periods of rest or low-intensity activity [12]. Used in athletics for decades to improve endurance performance [36], interest in the clinical applications of interval training has more recently gained significant attention [12]. Interval training can be defined in a variety of ways, varying in interval length and duration. Sprint interval training (SIT) involves Wingate style cycling, performing 4-7 sets of 30 second sprints at a supra-maximal workload separated by 4 minutes of recovery, for a total training session of 20 minutes [37, 38]. This form of interval training has been shown to significantly improve muscle oxidative capacity in as little as six sessions, or a total of ~15 minutes of exercise over the course of two weeks [37, 38]. Although efficient and effective, the supra-maximal nature of SIT makes it difficult for non-athletic populations to participate. A more practical model of interval training, defined as HIIT, involves more feasible work durations, ranging from 1-5 minutes at high intensity (80-110% of maximal capacity). This form of interval training, and its variant forms, have been shown to be a safe and effective method for improving cardiorespiratory fitness and cardiometabolic health outcomes in a variety of clinical populations [15, 39, 40].

A majority of research on HIIT has focused on the effects on cardiorespiratory fitness and skeletal muscle mitochondrial adaptations [11, 12, 41]. Both SIT and HIIT have been shown to elicit increases in cardiorespiratory fitness, mitochondrial density, and oxidative capacity that are comparable to moderate intensity continuous training (MICT), but achieved in 20% of the exercise time (30-60 minutes per week vs. 150 minutes per week, respectively) [33, 38, 42]. Skeletal muscle mitochondria serve as a primary regulator for substrate metabolism during submaximal exercise [41]. Increased mitochondrial density allows for greater fat oxidation, decreased reliance on carbohydrate/glycogen oxidation, and increased anaerobic threshold, supporting higher exercise intensity for a longer duration [41]. Metabolites associated with mitochondrial oxidation have been shown to be more pronounced with higher-intensity aerobic exercise compared to low-intensities [43]. Specifically, higher-intensity aerobic exercise was associated with significant increases in skeletal muscle concentrations of β-oxidation byproducts, primarily medium and long even-chain acylcarnitines [43]. This correlated strongly with abundance of mitochondrial enzymes, suggesting enhanced mitochondrial density and/or capacity [43]. Mitochondrial density is primarily regulated by the signaling proteins Ca2+/calmodulin-dependent protein kinase II (CaMKII) and AMP-activated protein kinase (AMPK), which in turn activates gene expression of peroxisome proliferator-activated receptor γ 1-α (PGC-1α) the primary regulator of mitochondrial biogenesis [41]. This process is initiated by elevated adenosine triphosphate (ATP) turnover, accumulation of metabolites, and production of reactive oxygen species [41].

During a single high-intensity bout lasting 30-60 seconds, ATP and phosphocreatine (PCr) stores are significantly reduced, and increased contribution from anaerobic glycolysis is required to maintain intensity [17, 44, 45]. Complete recovery of ATP/PCr stores can take up to 3-5 minutes, while complete recovery from anaerobic glycolysis may take an hour or more [17, 45]. Since recovery periods during a HIIT session only last one minute, ATP/PCr stores do not completely recover between exercise bouts and result in an increased dependence on anaerobic glycolysis and aerobic metabolism as the session progresses [45]. Depletion of ATP/PCr, in combination with increased hydrogen ion (H^+), lactate concentrations, and degradation of glycogen, creates significant metabolic disruption [45]. HIIT also

stimulates a significant catecholamine response, stimulating lipolysis from subcutaneous and intramuscular triglyceride stores [17]. This stimulates an increase in post-exercise oxygen consumption, energy expenditure, and fat oxidation, in order to restore homeostasis [45]. During a single exercise session, energy expenditure has been shown to be greater during MICT compared to HIIT, due to the longer duration of MICT [46]. However, energy expenditure has been shown to be significantly greater for up to 60 minutes following a HIIT session compared to MICT [16] with no differences in post-exercise energy expenditure between the two at 24-hours post exercise, despite MICT lasting twice as long and involving twice as much work [46]. Elevated catecholamine levels that occur with high-intensity exercise and HIIT, promote lipolysis and lipid oxidation, especially in the post exercise period [17, 47]. Respiratory exchange ratio (RER), an indirect measure of substrate oxidation, was significantly elevated immediately following a HIIT session compared to MICT, indicating greater carbohydrate oxidation during exercise and reflecting the more anaerobic/high intensity nature of HIIT [16]. However, at 30 and 60 minutes post-exercise, RER was significantly lower with HIIT training than MICT, suggesting HIIT favors greater fat oxidation in the post-exercise period [16]. These mechanisms may support improvements in body composition as a result of HIIT.

In a recent meta-analysis of 13 studies, Wewege et al. (2017) reported that HIIT reduced body fat by ~2 kg and waist circumference by ~3 cm over a 5-16 week time frame with varying protocols [48]. These losses were not different from MICT, however, it was emphasized that fat loss associated with HIIT was achieved in ~40% less training time [49, 50]. A second meta-analysis evaluating 31 studies ranging from 4-16 weeks reported similar findings, with HIIT/SIT reducing body fat by ~1.38 kg and percent body fat by ~1.26%, which was also not significantly different from MICT (-0.91 kg and -1.48%) even when matched for energy expenditure or workload [51]. Significant changes in body composition have been reported in interventions less than 4 weeks [13, 15]. After three weeks of HIIT significant reductions in body fat were reported in overweight and obese women (-1.96 ± 0.99 kg) [13]. This change is greater than 6- and 8-week interventions using a similar HIIT protocol in overweight and obese adults [34, 52]. Gillen et al (2013) reported an average of -0.6 kg for body fat, -0.75% for percent body fat, and -

0.06 kg for abdominal fat after 6-weeks [34]. Similarly, Sawyer et al. (2016), reported a significant decrease in percent body fat (-0.8%) after 3 days per week of HIIT, but a non-significant change in body fat (-0.9 kg) [52]. Differences could stem from baseline body fat percentages, with individuals with higher body fat at baseline potentially responding better [13, 53]. Differences could also be related to the sensitivity of the body fat measurement technique. Based on results of previous studies, interval training appears to be an effective and efficient method for reducing body fat, resulting in similar reductions in body fat compared to MICT, but in a significantly reduced amount of exercise time.

Due to the significant effects of interval training on mitochondrial and cardiorespiratory changes, research has focused primarily on weight loss and fat loss with HIIT. However, in analyzing body composition, a number of studies have also reported increases in LM after interval training. Heydari et al. (2012) reported significant increases in fat-free mass (1.2 kg) and increased LM in the legs and trunk in overweight men after 12-weeks of SIT [54]. After 6-weeks of SIT, MacPherson et al. (2011) reported a significant 0.6 kg increase in FFM in healthy, recreationally active college students. Gillen et al. (2013) reported a non-significant 0.6 kg average increase in total body LM, but significant increases in leg and gynoid region LM and in overweight and obese women after 6 weeks of HIIT [34]. After three weeks of HIIT, Smith-Ryan et al. (2015, 2016) reported an average 1.9 kg and 2.2 kg increase in LM in overweight and obese men and women, respectively [13, 15]. Although these increases in total body LM were non-significant, follow-up analysis showed a significant increase in muscle cross sectional area of the vastus lateralis [14]. In contrast, meta-analysis collectively demonstrated no significant effect of HIIT on LM and a non-significant, but greater magnitude of change in LM was recorded for HIIT compared to MICT [48].

Although aerobic based exercise is not associated with significant muscle hypertrophy [31], moderate intensity aerobic exercise has been shown to stimulate myofibrillar protein synthesis rates during early recovery, but rates return to baseline within 24-hours [55]. Similar to moderate-intensity aerobic exercise, myofibrillar protein synthetic rates were also elevated after a single bout of high-intensity aerobic exercise, but synthetic rates remained significantly elevated after 24 hours, suggesting a

potential effect of exercise intensity on muscle hypertrophy [55]. Muscle hypertrophy occurs when muscle protein synthesis exceeds muscle protein breakdown and contractile elements actin and myosin enlarge, adding sarcomeres in series or in parallel [56]. This process is mediated by a number of mechano-signaling pathways, including protein kinase B (Akt)/mammalian target of rapamycin (mTOR), mitogen-activated protein kinase (MAPK), and calcium-dependent pathways, that are initiated by mechanical tension, muscle damage, and metabolic stress [56]. Little is known about the mechanisms through which HIIT may induce muscle hypertrophy. Using resistance training induced hypertrophy as a model, it has been theorized that the increased force, power, and contraction intensity required during a HIIT session, increases recruitment of high-threshold motor units and mechanical tension, leading to increased activation of mTOR, the primary regulator of muscle hypertrophy [56]. In support of this theory, high-intensity aerobic exercise, but not moderate intensity exercise, has been shown to increase activation of mTOR, which significantly correlated with increased rates of myofibrillar protein synthesis rates following an exercise bout [55]. After two weeks of HIIT, increased peak torque of a maximal voluntary contraction (MVC), in addition to an increase in electromyography (EMG) amplitude and motor unit discharge rate at high force levels, were attributed to the high-intensity nature of HIIT compared to MICT [57]. Factors responsible for increases in maximal muscle strength include changes in muscle-fiber architecture, specifically muscle cross sectional area, and increased muscle activation [57, 58]. Although specific changes in muscle architecture with HIIT training have not been evaluated, increased muscle cross sectional area has been reported in overweight and obese adults after three weeks of HIIT [14]. It has also been suggested that the high, rapid contraction intensity of HIIT may damage contractile elements, inducing an acute inflammatory response, stimulating satellite cell repair and subsequent hypertrophy [18, 59]. Finally, the production of anaerobic metabolites may mediate the hypertrophic response, potentially increasing activity of anabolic transcription factors, increasing muscle fiber damage, and stimulating sympathetic nerve activation [56].

Metabolically, the maintenance of a greater LM could also have implications for fat loss [60]. Based on metabolic profiling, high-intensity aerobic exercise is associated with increased muscle

concentrations of several amino acids, most notably the branched-chain amino acids (BCAA) [43]. It was speculated that in the context of exercise training, amino acids may be diverted towards muscle protein synthesis rather mitochondrial energy production [43]. Muscle protein turnover, or the rate of muscle protein synthesis and breakdown, is one of the more variable components of resting energy expenditure, and greater LM significantly increases resting metabolic rate [60]. Muscle protein turnover is also primarily fueled by fat oxidation, which could contribute to decreases in body fat [60, 61].

PROTEIN SUPPLEMENTATION

The effects of exercise alone on weight loss and body composition changes are typically unremarkable, but when combined with a nutritional intervention (i.e. caloric restriction and/or macronutrient manipulation) changes become much more pronounced [62, 63]. High-protein diets have been shown to be especially beneficial for positively altering body composition, supporting metabolically active lean tissue, while also promoting significant decreases in body fat [3, 5, 64-66]. High-protein diets are loosely defined as providing ≥25% of total energy intake from protein (PRO) or above 2.4 $g \cdot kg^{-1}$; commonly coupled with a reduction in carbohydrate (CHO) intake (40-50% of energy intake) [65]. Protein intakes of 1.2 – 1.6 $g \cdot kg^{-1} \cdot d^{-1}$ have been shown to promote positive physiologic and metabolic responses in individuals with type 2 diabetes and metabolic syndrome, while intakes of 1.2 – 2.4 $g \cdot kg^{-1} \cdot d^{-1}$ are recommended to promote recovery and adaptation from endurance and strength training [8, 9]. The primary role of dietary protein is to provide amino acids that are essential for building structural proteins in the body, but protein also has many metabolic roles, most notably, stimulation of muscle protein synthesis [66, 67].

Muscle protein is in a constant state of turnover, fluctuating between states of muscle protein synthesis (MPS) and muscle protein breakdown (MPB) [66]. Exercise, especially resistance training, stimulates MPS, but also increases MPB, resulting in a reduction in the balance between synthesis and breakdown [19, 66]. In a fasted state, however, synthesis does not exceed breakdown and net protein balance remains negative [19, 66]. Protein, specifically essential amino acids (EAA), are a potent

stimulator of MPS, stimulating a positive protein balance [19, 66]. When EAAs are consumed prior to or following exercise, the positive protein balance is augmented [19, 66, 68-70].

There are numerous studies supporting the beneficial effects of PRO intake with exercise on body composition and metabolism. In a classic study by Layman et al. (2003), overweight and obese women who consumed diets with a lower CHO:PRO ratio (1.4:1; 171 g CHO and 125 g PRO per day) lost more body weight, specifically more body fat (-5.6 kg vs. -4.74 kg) and less LM (-0.88 kg vs. -1.21 kg), compared to women who consumed a higher CHO:PRO ratio diet (3.5:1; 239 g CHO and 68 g PRO per day) [64]. When combined with an aerobic and resistance training program designed to meet physical activity recommendations, women consuming the high-protein diet lost even greater body fat (-8.8 kg) and minimized loss of LM (-0.4 kg) [5]. In a study by Arciero et al. (2014), overweight and obese adults who consumed 20g of whey protein 3 times per day combined with a multimodal exercise program that included interval training, lost significantly more body fat (-2.8 kg vs. -1.0 kg), gained a greater percentage of LM (2.0% vs. 0.6%), and improved indicators of insulin sensitivity, compared to a PRO only group [3]. When combined with 6 days per week of a combined resistance and HIIT exercise program, Longland et al. (2016) showed that consumption of 1.2 $g \cdot kg^{-1} \cdot d^{-1}$ of PRO was effective for maintaining LM and promoting fat loss, while 2.4 $g \cdot kg^{-1} \cdot d^{-1}$ of PRO was effective for increasing LM (+1.2 kg), despite a significant caloric deficit (40% reduction) [6]. Both groups also improved strength, aerobic, and anaerobic performance outcomes [6].

Few studies have directly evaluated how nutrient intake influences the unique metabolic adaptations of HIIT. Gibala et al. (2014) proposed that nutrition may improve energy metabolism during HIIT, which could facilitate greater total work during a HIIT session, subsequently enhancing the training stimulus [11]. Appropriate nutrition would also support the recovery process from HIIT, leading to enhanced physiological adaptations over time [11]. Studies in elite athletes have shown that training in a carbohydrate restricted/glycogen depleted state, withholding CHO during exercise, and/or delaying CHO intake/glycogen resynthesis after exercise, significantly enhances cell signaling pathways and upregulates oxidative enzymes that lead to increased total body and intramuscular lipid oxidation [71, 72]. Although

this has not been shown to benefit performance, this 'train low' approach enhances the catecholamine response to a high intensity workout, stimulating greater fat and intramuscular lipolysis, and increases stimulation of AMPK, p38 MAPK, and PGC-1α, the same mechanisms upregulated by HIIT that significantly increasing mitochondrial biogenesis and oxidative capacity [71, 73]. While CHO consumption in these studies was shown to blunt the stimulation of key signaling pathways associated with mitochondrial adaptation, PRO consumption did not attenuate signaling, and it was recommended that 20-25g of protein be consumed before, during, and/or after exercise in order to maintain protein balance and support muscular recovery [71, 73]. These studies suggest that protein consumption, rather than CHO consumption, around a HIIT session may enhance mitochondrial biogenesis and fat oxidation while also supporting MPS.

In addition to supporting mitochondrial and muscular adaptation, protein consumption prior to a HIIT bout has been shown to significantly increase post-exercise energy expenditure and fat oxidation, to a greater degree than CHO consumption [16]. Protein has a higher thermic effect of feeding compared to carbohydrate and fat, which can contribute to a greater energy expenditure [74, 75]. Protein intake would also stimulate greater muscle protein turnover, increasing energy expenditure and fat oxidation associated with maintenance of LM, as previously described [60, 61].

SEX DIFFERENCES

When evaluating individual responses to an exercise training program, there is considerable variability in body composition changes [76, 77]. In a study evaluating body composition changes in overweight and obese adults in response to MICT, ~23% of individuals gained weight over the course of the 10-month intervention [76]. In a follow-up analysis, males and females who lost <5% body weight (exercise non-responder) had no changes in FM (Males: +0.3±2.3 kg; Females: -1.2±4.8 kg) or fat-free mass (Males: +0.3±1.8 kg; Females: -0.6±5.3 kg). Male non-responders were found to have increased energy intake and decreased non-exercise energy expenditure, compared to those who lost ≥5% body weight [77]. However, no differences in energy intake or energy expenditure were found between females

who lost weight and those who did not, suggesting other non-lifestyle mechanisms may influence the female response to exercise [77]. In a strains of mice bred to respond negatively to exercise (i.e. gain weight), males responded similarly to MICT and HIIT, while females gained fat after a MICT program, but lost fat after HIIT; suggesting that sex and genetic background can influence response to exercise [78].

There is considerable debate as to whether males and females respond differently to HIIT. Males have been reported to have greater increases in cardiorespiratory fitness [79], fat loss [80, 81], increased mixed muscle protein synthesis [35], and improved metabolic outcomes, notably improved glycemic control [82, 83], compared to females. In contrast, one study reported females to have greater improvements in cardiorespiratory fitness [81], while a majority of studies report no effect of sex on the cardiorespiratory [35, 82-85], body composition [81, 85], or metabolic [81, 84-88] effects of HIIT. Differences in response to HIIT training have been attributed to males having greater glycogen breakdown during sprints, greater anaerobic capacity, greater portion of type II fibers, and a greater catecholamine response than women [11]. Despite these suggested differences, very few studies directly address sex differences in response to HIIT [35, 79, 81, 84, 85, 87], with only two evaluating differences in body composition responses [81, 85]. Using a 3-site skinfold model, Astorino et al. (2011) reported no changes in percent body fat in men (-0.3%) or women (+0.2%) after six sessions of SIT over the course of 2-3 weeks [85]. Using dual-energy x-ray absorptiometry, Bagley et al (2016) reported that men lost a greater percentage of FM (-1.5%) and trunk fat (-0.7kg), than women (FM: -0.1%; trunk fat: -0.1 kg), after 12-weeks of SIT [81]. Men also tended to gain more LM (+0.7 kg) than women (+0.1 kg) [81].

Metabolically, females rely more heavily on aerobic metabolism during exercise, oxidizing more fat and less CHO than men, who show a greater capacity for anaerobic metabolism [89]. Sex differences in metabolism are primarily attributed to the influence of estrogen, or the lack thereof [89]. Mechanisms by which estrogen affects metabolism across different tissues is not well understood, but a recent study suggests that 17 β-estradiol may integrate in the inner mitochondrial membrane, decreasing membrane viscosity, and subsequently increase oxidative capacity, cell redox balance, and improve insulin

sensitivity [90]. It was further suggested that these effects may be tissue specific, with a greater impact on skeletal muscle mitochondria [90], which would have important implications for substrate metabolism during exercise. Other studies in overiectomized rats have shown that estradiol treatment stimulates mitochondrial biogenesis and oxidative capacity [90, 91], while estradiol treatment in male rats reduced glycogen utilization, increased lipid availability, and improved 2 hour exercise running performance [92]. In humans, post-menopausal women taking hormone replacement therapy had greater improvements in insulin sensitivity with exercise training compared to post-menopausal women not taking hormone replacement therapy [93]. In exercising men, eight days of 17 β-estradiol supplementation reduced CHO utilization and increased fat utilization, suggesting that estradiol influences substrate metabolism during exercise [94].

Due to the divergent sex-based responses in substrate metabolism, there has been increasing interest in tailoring nutritional approaches for males and females in order to maximize athletic performance and health. An important meta-analysis demonstrated lower rates of fat oxidation in females, compared to males following fasted exercise; with females yielding greater fat oxidation rates following fed exercise [95]. Traditionally, high-carbohydrate consumption has been recommended in order to maximize glycogen stores and fuel high-intensity exercise [96]. However, in a cohort of women, protein consumption prior to exercise reduced post-exercise RER and increased post-exercise metabolic rate to a greater degree than CHO, suggesting protein consumption prior to exercise promotes greater fat oxidation and energy expenditure post-exercise in women [16]. In the same study, HIIT also increased post-exercise RER and increased post-exercise metabolic rate to a greater degree than MICT or high-intensity resistance training [16]. Other studies have shown that protein intake supports metabolic flexibility [97] and improved cardiometabolic outcomes [4, 64] in women. Over time, protein supplementation and HIIT could lead to more notable changes in body composition in women.

CONCLUSION

High intensity interval training significantly improves cardiorespiratory fitness and oxidative capacity in both men and women, and may be an efficient and effective approach for improving body composition. Fat reduction as a result of HIIT has been shown to be similar to MICT, but in a significantly reduced amount of exercise time [41]. Increases in LM, in combination with decreased body fat, have been reported with HIIT in a few as three weeks or nine sessions of HIIT [13, 15]. Body composition changes as a result of HIIT could have significant health benefits, but more research is needed to understand the mechanisms through which HIIT may be supporting hypertrophy. Protein supplementation, in combination with HIIT training, may promote even greater changes in body composition, stimulating MPS, while metabolically, enhancing mitochondrial biogenesis and fat oxidation. Finally, further research is needed to understand how sex may modulate responses to HIIT and protein supplementation.

CHAPTER III

METHODOLOGY

EXPERIMENTAL DESIGN

Using a 2:2:2:1 block randomized design, participants were randomized, to one of four, eight-week intervention groups: 1) HIIT, two days per week of cycle ergometry; 2) essential amino acids (EAA) supplementation (7.2 grams EAA daily); 3) HIIT+EAA; or 4) control (CON), no intervention maintaining normal diet and exercise habits (Figure 2). Measurements of body composition, metabolic rate, substrate metabolism, and cardiorespiratory fitness were measured at baseline, 4weeks, and 8weeks; cardiometabolic markers and metabolomic markers were measured at baseline and 8weeks. Whole-body protein turnover was measured in a subsample of individuals from the HIIT (N=8), EAA (N=7), and HIIT+EAA (N=7) groups at baseline and 8weeks.

Prior to enrollment, all participants completed a phone screening for inclusion/exclusion criteria. Those determined to be eligible based on the phone screening completed an in-person enrollment visit in which they provided written informed consent, completed a health history questionnaire to confirm inclusion/exclusion criteria, and underwent a 12-lead electrocardiogram (EKG). Women completed a urine pregnancy test to confirm negative status. Participants were asked to arrive to testing sessions following a 12 hour fast, consuming no food, caffeine, or alcohol. Participants were also be asked to abstain from physical activity for 24 hours prior to testing.

SUBJECTS

An original 651 individuals expressed interest and were sent initial information about the study. Of those who initially expressed interest, 37 declined, 194 were excluded for not meeting inclusion

criteria (44 of whom were excluded during a full telephone screening), and 331 did not respond to the initial contact or lost to follow-up, resulting in 89 individuals who met initial inclusion criteria and completed an in-person enrollment visit. At the enrollment visit, five individuals were excluded for reasons related to exceeding exercise criteria (N=2), pregnant (N=1), and BMI too high (N=2). This resulted in 84 individuals who were randomized to one of the four intervention arms and scheduled for baseline testing. Fourteen individuals did not return for baseline testing, for reasons related to starting medication (N=1), pregnancy (N=1), withdraw for personal reasons (N=3), and lost to follow-up (N=9); four individuals completed baseline testing, but dropped out before completing mid-or post-testing due to sickness (N=2) and lack of time (N=1), and were excluded from the final analysis. Full CONSORT information is reported in figure 1.

Sixty-six overweight and obese men (N=33) and women (N=33), 25 – 50 years participated in the current study (Race: 69% White, 13% Black, 4% Hispanic, 3% Asian, 11% Two or more races; Age: 36.7 ± 6.0 years; Height: 171.4 ± 9.8cm; Weight: 94.5 ± 14.7 kg; %BF: 36.0 ± 7.8%). Overweight and obese was defined as a body mass index (BMI) of 28 – 40 $kg \cdot m^{-2}$ and/or percent body fat (%BF) ≥ 25% for men, and BMI of 25 – 40 $kg \cdot m^{-2}$ and/or %BF ≥ 30% for women [21] determined by measured height (stadiometer; Perspective Enterprises, Portage, MI, USA) and weight (mechanical scale; InBody770, BioSpace, Seoul, South Korea) and %BF from bioelectrical impedance analysis (InBody770, BioSpace, Seoul, South Korea), respectively. Women were eumenorrheic, determined as reporting consistent menstruation for the three months prior to enrollment, and confirmed not-pregnant by a urine HCG pregnancy test. Participants were otherwise healthy, non-smokers, who participated in less than 150 minutes per week of moderate exercise, less than two days per week of resistance training, and had not participated in HIIT in the 12 weeks prior to enrollment. Individuals were excluded from participation if they: 1) had current and/or history of cardiovascular disease, diabetes, metabolic, thyroid, pulmonary, renal, hepatic, gastrointestinal, musculoskeletal disorders or medical or surgical events, such as bariatric surgery, heart surgery, or any joint or musculoskeletal surgeries occurring the 6-months prior to enrollment; 2) had uncontrolled hypertension or an abnormal electrocardiogram; 3) has a diagnosed

mental disorder; 4) were taking medications inconsistently (i.e. blood pressure medication, anti-depressants, anti-anxiety, hormonal contraceptives) or were taking a medication that could influence primary study outcomes (i.e. metformin, insulin, thyroid); 5) had lost or gained greater than eight pounds within three months prior to enrollment; 6) were consuming a high protein diet, defined as consuming $\geq$1.6 $g \cdot kg^{-1} \cdot day^{-1}$ [65]; 7) were consuming meal replacements or dietary supplements within eight weeks prior to enrollment, specifically protein, creatine, beta-alanine, carnosine, taurine, or beta-hydroxy beta-methylbutyrate; 8) had known sensitivity to the EAA supplement; 9) participated in another clinical trial that may influence study outcomes within four weeks prior to enrollment; 10) had severely impaired hearing or speech or inability to speak English; 11) were unwilling or unable to comply with the study protocol, including abstaining from food and caloric beverages (12 hrs), caffeine (12 hrs), alcohol (24 hrs), and physical activity (24 hrs) prior to testing days.

PROCEDURES

Body Composition

A four compartment (4C) model, previously validated by our laboratory (Equation 1), were used to estimate fat mass (FM), percent body fat (%BF), and fat-free mass (FFM) [98]. Components of this equation include: 1) body volume (Equation 2), derived from a dual-energy x-ray absorptiometry total body scan (DXA; GE Lunar iDXA, GE Medical Systems Ultrasound & Primary Care Diagnostics, Madison, WI, USA) [98]; 2) total body water, measured using multi-frequency bioelectrical impedance spectroscopy (BIS; SFB7, ImpediMed, Queensland, Australia); and 3) total body bone mineral density (Mo; Equation 3), calculated using total body bone mineral content (BMC), measured from the DXA.

Equation 1: FM (kg) = 2.748(BV) – 0.699(TBW) + 1.129(Mo) – 2.051(BM)

%BF = (FM/BM) × 100

FFM (kg) = BM – FM

Equation 2: $\text{BV (L)} = \frac{FM}{0.84} + \frac{LM}{1.03} + \frac{BMC}{11.63} + (-3.12)$

Equation 3: Mo = BMC × 1.0436

Test re-test reliability for the 4C model from our laboratory is as follows: FM (intraclass correlation coefficient (ICC)=0.995, standard error of measure (SEM)=0.831 kg, minimum difference (MD)=2.30 kg); %BF (ICC=0.982, SEM=0.960%, MD=2.6%); and FFM (ICC=0.996, SEM=0.999 kg, MD=2.75 kg).

Dual-energy x-ray absorptiometry

Prior to scanning, subjects will be asked to remove all metal, plastics, and heavy clothing, wearing only lightweight athletic clothing. Subjects were positioned in a supine position in the center of the scanning table, with arms and legs inside the scanning parameter. Participants larger than the scanning area were positioned such that the entire right side of the body was inside the scanning parameter, with as much of the remaining body inside the scanning area as possible. Composition of left limbs outside of the scanning parameter was then estimated from the right side. All DXA scans were performed by a trained technician, following manufacturer guidelines. All scans will were analyzed using manufacturer software (enCORE Software Version 16). For sub-analysis of segmental composition of the thigh, a region-of-interest (ROI) was manually drawn such that, 1) the thigh was separated from trunk by a line bisecting the femoral head and touching the ischial tuberosity, as would be drawn to form the pelvic triangle; and 2) the thigh was separated from the lower shank by a line drawn bisecting the intercondylar space between the femur and the tibia. Visceral adipose tissue (VAT) mass (kg^2) and volume (cm^3) was quantified from the pre-defined android ROI set by DXA software. This region is defined as the area spanning 20% of the distance from the top of the iliac crest to the base of the skull [99]. Test-retest reliability for VAT mass measurements from our lab are as follows: Mass (ICC=0.98, SEM=0.11 kg, and MD=0.22 kg) and volume (ICC=0.98, SEM=118.73 cm^3, and MD=233.85 cm^3).

Bioelectrical impedance spectroscopy

While lying supine on a table with separation between the limbs, leads were connected to four electrodes placed on the right wrist (bisecting the ulnar head), five centimeters distally on the hand, the right ankle (bisecting the malleoli), and five centimeters distally on the foot. The average of two measurements was recorded for TBW, intracellular fluid (ICF), and extracellular fluid (ECF).

Muscle Characteristics

Muscle cross-sectional area (mCSA) of the vastus lateralis (VL), rectus femoris (RF), and vastus medialis (VM) was determined from panoramic ultrasound (US) scans of the thigh (GE LOGIQ-e, Software version R8.0.7, GE Healthcare, Wisconsin, USA) using a linear array US transducer prode (GE: 12L-RS) and standardized frequency (10 Hz) and gain (50) settings [22, 23]. Measurements were made by applying the device probe directly against the skin at the peak anatomical cross-sectional area of each muscle, defined as 30%, 50%, and 60% of femur length for the VM, VL, and RF, respectively.

Pennation angle (PA) and fascicle length (FL) of the VL were evaluated from panoramic scans along the fascicle plane at 50% of femur length [26]; muscle volume (mV) was evaluated from cross-sectional scans of the VL taken at 25%, 50%, and 75% of muscle length [mV = (25% muscle length (cm) × 25%mCSA (cm^2)) + (25% muscle length (cm) × 50%mCSA (cm^2)) + (25% muscle length (cm) × 75%mCSA (cm^2))] [27, 100]. The scans were performed by the same technician while the subject lay supine with the right leg extended and relaxed on the examination table for approximately 5 minutes.

Muscle cross-sectional area was determined by tracing the outline of the muscle along the inside fascial border [22, 23]. Echo intensity (EI), was determined using grayscale analysis, with a correction for subcutaneous fat thickness [$EI = EI_{raw} + (SAT \times 40.5278)$] from the cross sectional image of the VL taken at 50% of femur length [22, 23, 101]. Fascicle length was determined as the length of the fascicle between the superficial and deep aponeuroses [26]; pennation angle was determined by measuring the angle between the deep aponeurosis and the same fascicle used to determine FL [26]. The same

technician performed all analysis for each outcome. All images will be exported and analyzed using Image-J software (National Institutes of Health, USA, version 1.51). Each image was individually calibrated for analysis by measuring the number of pixels in a known distance (image depth). Two images from each location were analyzed and an average of the two measures was reported. Test-retest reliability for mCSA and EI from our lab are as follows: mCSA ICC=0.99, SEM of 0.744 cm^2; EI ICC=0.99, SEM=1.5 a.u.

Total Body Protein Turnover

Whole body protein turnover (g N/24hr) was be determined by [^{15}N]alanine isotope tracer (98% enriched, Cambridge Isotope Lab, Andover, MA) [102], in which participants ingested a 2.00 gram dose of [^{15}N]alanine mixed with water. For the 24hrs following ingestion, participants were asked to collect urine from all voids and keep a diet record of all food and drink consumed. Diet records were analyzed for protein intake (g) to account for dietary nitrogen intake. A zero and 24-hour blood draw was collected to measure blood urea nitrogen. Isotopically labeled nitrogen from the urine samples was used to determine nitrogen flux according to Fern et al. [103]. Total body protein synthesis (PS) and breakdown (PB) was calculated from urine samples according to Stein et al. [104] and used to determine net protein balance and flux. Samples were analyzed at the Center for Translational Research in Aging and Longevity, University of Arkansas Medical Sciences, Little Rock, AR.

Resting Metabolic Rate and Substrate Metabolism

Resting metabolic rate (RMR) and respiratory exchange ratio (RER) were evaluated using a ventilated canopy with indirect calorimetry. Respiratory gases, oxygen uptake, and carbon dioxide production, were analyzed over 30 second intervals with a metabolic cart (TrueOne 2400, ParvoMedics, Inc., Sandy, UT) for 30 minutes while lying in a supine position. The percentage of carbon dioxide was maintained between 1.0 – 1.2%, with the first five minutes of the test discarded to allow for gas to normalization; RMR and RER were averaged over the remaining 25 minutes of the test. Test-retest

reliability from our lab are as follows: RMR (ICC=0.94, SEM=125.6 kcal·day^{-2}, MD=244.3 kcal·day^{-2}) and RER (ICC=0.83, SEM=0.03 arbitrary units (a.u.), MD=0.05 a.u.).

Cardiometabolic Blood Markers and Metabolomics

Fasting blood glucose, total cholesterol, triglycerides, HDL-cholesterol, non-HDL were measured from a 4 ml blood sample, immediately analyzed using an Alere Cholestech LDX® Analyzer. Serum from a 4 ml blood sample was separated and analyzed for insulin using established enzymatic assays. Fasting EDTA plasma from another 4 ml blood sample was analyzed for circulating metabolites using a targeted mass spectrometry-based platform [105, 106]; these include fasting concentrations of branched-chain amino acids (leucine, isoleucine, valine) and acylcarnitines [43, 107] (Appendix 1). All blood draws were done in the Applied Physiology Lab by an individual trained in phlebotomy.

Cardiorespiratory Fitness

Peak oxygen consumption (VO_2peak) and ventilatory threshold (VT) were determined from a ramp-based exercise test on an electronically braked cycle ergometer (Corival Lode, Gronigen, The Netherlands). Following a two-minute warm-up at 20watts (W), intensity increased 1W every 3 seconds until volitional fatigue; participants were instructed to maintain a pedal cadence between 60-80 rpms for the entirety of the test. Respiratory gases were analyzed breath-by-breath using indirect calorimetry (Parvo Medics TrueMax 2400®, Salt Lake City, UT); the three highest oxygen consumption values were averaged and recorded as VO_2peak. In accordance with pre-established criteria [108], the test was considered maximal if it met a minimum of two of the following criteria: a plateau or heart rate within 10% of age-predicated HR max; a plateau or increase of no more than 150 ml/min in VO_2; or achieved an RER >1.15. Ventilatory threshold (VT) was determined as the intersection point of two linear regression lines fitted to the upper and lower portion of the ventilation versus VO_2 curve using manufacturer software (True One 2400® Metabolic Measurement System, Parvo-Medics Inc., Provo UT) [109]. Heart rate (HR) was monitored throughout the test (Polar Electro Inc., Lake Success, NY); the highest HR

achieved during the test was recorded as the max HR. The highest wattage achieved during the exercise test was used to set an appropriate individualized training load for the start of the HIIT protocol.

Dietary Intake

Subjects were asked to complete three-day dietary logs at baseline, 4weeks, and 8weeks to account for the influence of normal dietary intake. Subjects were instructed to record all food and drink consumed on two, non-consecutive weekdays and one weekend day. Subjects were given detailed verbal and printed instructions on how to complete the diet logs and estimate portion sizes. Instructions included: recording the amount of food, drink, gum, candy, condiments, supplements, etc. as consumed at each meal and snack throughout the day and providing as much detail about portion size, brand names, and preparation techniques as possible. Diet logs analyzed for average calories (CAL; kcal), carbohydrate (CHO; g), fat (FAT; g), and protein (PRO; g) using nutrition analysis software (The Food Processor, version 10.12.0, Esha Research, Salem, OR, USA). To account for potential misreporting of dietary information that can occur with self-reported dietary intake, structured 24hr dietary recalls were also conducted at the baseline and 8-week visits, using a modified multiple pass method [110]. Recalls were conducted in-person by the same researcher. Participants were asked to recall, without interruption, everything they ate, drank, or consumed for the day prior, starting from when they first started eating until they started fasting for the study visit. Participants were then read back what they reported, being asked follow-up questions about food type and amounts consumed as necessary. Participants were then asked about consumption of commonly forgotten foods, including: beverages (coffee, tea, soft drinks, milk or juice), alcoholic beverages, sweets (cookies, candy, ice cream, or other sweets), snacks (chips, crackers, popcorn, pretzels, nuts, or other snack foods), fruits, vegetables, cheese, and breads (breads, rolls and tortillas).

High-Intensity Interval Training

Those assigned to participate in HIIT, trained two days per week for eight weeks. All training occurred on a cycle ergometer (Corival Lode, Gronigen, The Netherlands), in the Applied Physiology Lab on the UNC campus, with one-on-one supervision from trained research personnel. Each session consisted of a self-selected warm-up (≤5 minutes), followed by alternating sets of one minute of pedaling at a resistance that corresponded with 90% max wattage and one-minute recovery at complete rest (Figure 3A). After each interval, HR was recorded and subjects were asked to rate their exertion using a Borg Rating of Perceived Exertion scale. Individuals started with six sets of intervals, adding one additional set each week until reaching 10 sets at week five and maintaining 10 sets for the remainder of the 8 weeks (Figure 3B). On the last set of each session, individuals were asked to ride as long as possible. If the individual was able to ride for an additional 15 seconds (75 seconds total), resistance was increased by 7% at the next session to maintain an appropriate individualized high intensity workload (based on unpublished pilot data). If the individual did not ride for longer than 15 seconds, the resistance was maintained for the next session. At least 24-hours separated training sessions. Intensity was individualized for each participant based on maximum wattage reached during baseline cardiovascular fitness (VO_2peak) testing. Completion of at least 13 sessions was considered compliant.

Essential Amino Acid Supplementation

Those assigned to an EAA supplementation group were instructed to consume an EAA powder mixed with water (8-12 oz), two times per day (REAAL, Twinlab Corporation, Hauppauge, NY, USA). One serving of the powder contained 3.6 g of a patented-ratio blend of L-leucine, L-lysine HCl, L-valine, L-isoleucine, L-arginine, L-threonine, L-phenylalanine, L-methionine, L-histidine, and L-tryptophan, formulated to support muscle growth. Participants were instructed to consume the supplement between meals; one serving between the hours of 9:00am – 12:00pm and the second serving between the hours of 3:00pm – 11:00pm, with at least 3 hours separating doses. For participants randomized to the HIIT+EAA group, the EAA supplement was consumed 30 minutes prior to- and 30 minutes after the HIIT session on

training days, provided by the study staff. All participants were given a log to record supplement consumption at home. Empty tubs were returned and collected at 4weeks and 8weeks to track compliance. 80% consumption (g) was considered compliant.

STATISTICAL ANALYSIS

Sample Size Determination

A priori power calculations: When considering multiple effect size calculations (F=0.129-0.423) [111] from between group comparisons of changes in FM (kg), LM (kg), mCSA (cm^2), and %BF following HIIT, protein supplementation, or a combination of exercise and protein supplementation [3, 13-15], a sample size of 70 was considered sufficient to achieve a power of 0.8, for four groups (HIIT, EAA, HIIT+EAA, CON) and three measures (base, 4wk, 8wk), with a conservative correlation of 0.5 among repeated measures, a nonsphericity correction ϵ of 1, and a significance level of 0.05. In order to account for at least 10% dropout and maintain equal male/female representation in each intervention group, 78 participants were aimed to be enrolled. Power calculation were conducted using G-Power version 3.1.9.2, with an ANOVA: repeated measures, within-between interactions F-test.

Statistical Procedures

A modified intent-to-treat analysis was conducted, including only participants who completed mid- (N=66) and/or post-testing (N=62). Adherence was evaluated based on number of sessions completed and/or total grams of EAA supplement consumed.

Manuscript 1: Group-by-time interaction effects on body composition (FM, LM, %BF, VAT), metabolic rate, substrate utilization, and cardiorespiratory fitness were evaluated using separate 4 × 2 [group (EAA vs. HIIT vs. HIIT+EAA vs. CON) × time (4week vs. 8 week)] mixed factorial ANCOVAs, covaried for baseline values. Differences in cardiometabolic markers between groups at 8weeks were evaluated using one-way ANCOVAs [group (EAA vs. HIIT vs. HIIT+EAA vs. CON) × time (8 week)], covaried for

baseline values. In the absence of a significant interaction effect, the interaction term was removed from the model to evaluate simple main effects. One-way repeated measures ANOVAs were used to evaluate simple main effects for time; one-way between-subject ANOVAs were used to evaluate simple main effects for group. Significant one-way ANOVAs were followed by pairwise t-tests using Bonferroni corrections for multiple comparisons. 95% confidence intervals (95% CI) on mean change scores adjusted for baseline values were also completed to assess changes from 0-4weeks, 4-8weeks, and 0-8weeks. If the 95% CI included zero, the mean change score was not considered statistically significant or no statistically significant change ($p>0.05$). If the 95% CI interval did not include zero, the mean change score was considered statistically significant ($p \leq 0.05$).

Group-by-time-by-sex interaction effects on body composition, metabolic rate, substrate utilization, and cardiorespiratory fitness were evaluated using separate 4 × 2 × 2 [group (EAA vs. HIIT vs. HIIT+EAA vs. CON) × time (4week vs. 8 week) × sex (male vs. female)] mixed factorial ANCOVAs, covaried for baseline values, using the same procedures as described for full group effects. Group-by-sex differences in cardiometabolic markers at 8weeks were evaluated using 4 × 2 mixed factorial ANCOVAs [group × sex], covaried for baseline values. 95% CI were also completed to assess changes from 0-4weeks, 4-8weeks, and 0-8weeks.

Manuscript 2: Using the same procedures as described for manuscript 1, group by time interaction effects on total body LM and thighLM, were evaluated using separate 4 × 2 [group (EAA vs. HIIT vs. HIIT+EAA vs. CON) × time (4week vs. 8 week)] mixed factorial ANCOVA's, covaried for baseline values. Secondary outcomes including muscle size (mCSA, MV), quality (EI), and architecture characteristics (FL, PA) were also evaluated using 4 × 2 mixed factorial ANCOVA's, covaried for baseline values. Differences in whole-body protein turnover between groups (HIIT vs. EAA vs. HIIT+EAA) at 8weeks were evaluated using one-way ANCOVAs, covaried for baseline values. Group by time by sex interaction effects on total body LM, thighLM, and muscle size, quality, and architecture

characteristics were evaluated using separate 4 × 2 × 2 (group × time × sex) mixed factorial ANCOVA's, covaried for baseline values.

All statistical computations were performed using SPSS (Version 21, IBM, Armonk, NY, USA), using an $\alpha = 0.05$ to determine statistical significance.

CHAPTER IV

MANUSCRIPT 1

BODY COMPOSITION AND METABOLIC EFFECTS OF HIGH-INTENSITY INTERVAL TRAINING AND ESSENTAIL AMINO ACID SUPPLEMENTATION

INTRODUCTION

Obesity is associated with a myriad of metabolic health complications partially attributed to dysregulation of skeletal muscle metabolism [60, 82]. Disruption in skeletal muscle oxidative capacity is an underlying factor in the development of insulin resistance, metabolic syndrome, and cardiovascular disease [12, 60, 112]. Physical activity is essential for maintaining skeletal muscle health and reducing cardiometabolic disease risk [1, 2]. Despite known benefits, more than half of adults do not meet the recommended minimum of 150 min of moderate intensity exercise [10], creating a need for more sustainable approaches to exercise for the improvement of metabolic health.

High-intensity interval training (HIIT) has been shown to promote significant improvements in cardiorespiratory fitness and metabolic health, comparable to moderate continuous exercise, but in a significantly shorter amount of time and reduced exercise volume [11, 12]. HIIT is broadly defined as repeated bouts of near maximal (~90%) exercise lasting ~60 seconds, interspersed with periods of rest or low intensity exercise. This has been shown to be a feasible and enjoyable option for a variety of clinical populations, including overweight and obese [12, 113]. Prior research on HIIT training has largely focused on the rapid cardiorespiratory and mitochondrial adaptations [12], but there is increasing interest in the effect on body composition. Results of meta-analyses suggest that that HIIT is just as effective as moderate intensity exercise for reducing body fat, but achieved in 40% less training time [48, 51]. In addition to fat loss, increases in lean mass (LM) and muscle size have also been reported as a result of HIIT [14, 15, 34, 48, 51, 80, 114, 115]. Significant increases in muscle hypertrophy are typically

associated with resistance exercise, but myofibrillar protein synthesis rates have been shown to be increased for up to 48 hours following a high-intensity exercise session [18, 55], suggesting HIIT could stimulate muscle hypertrophy. Simultaneous fat loss and muscle gain, in combination with an increase in cardiorespiratory fitness would have significant health implications.

Nutritional support, specifically from protein, is needed to support increases in LM. The stimulatory effects of protein on muscle protein synthesis are primarily driven by essential amino acids (EAA) [116, 117]. Free-form EAA supplementation has been shown to stimulate a greater anabolic response compared to a mixed meal or whey protein in recreationally active men [117]. Ingestion of 6g of EAA has also been shown to effectively increase muscle protein balance following a bout of resistance exercise [116]. Although this small dose effectively stimulated an increase in muscle protein when combined with resistance exercise, the effect when combined with HIIT has not yet been evaluated [116, 117].

Preliminary research from our lab has demonstrated that consumption of protein prior to a HIIT session augments post-exercise energy expenditure and fat oxidation, compared to carbohydrate [16]. These results suggest a potential synergistic effect of HIIT and protein, but whether these results translate to changes in body composition are unclear. There are also known differences in substrate metabolism between men and women at rest and during exercise, with women showing a greater preference for fat oxidation, while men are more efficient at glucose metabolism [20]. Since HIIT and high protein diets have separately resulted in improved lipid oxidation, HIIT combined with EAA supplementation may create a more favorable metabolic environment to support weight loss, particularly in women. The purpose of this study aimed to compare the independent and combined effects of eight weeks of HIIT and EAA supplementation on body composition and total body metabolism in overweight and obese men and women; an exploratory purpose was to explore the modulatory effects of sex.

METHODS:

Participants

An original 651 individuals expressed interest and were sent initial information about the study. Of those who initially expressed interest, 37 declined, 194 were excluded for not meeting inclusion criteria (44 of whom were excluded during a full telephone screening), and 331 did not respond to the initial contact or lost to follow-up, resulting in 89 individuals who met initial inclusion criteria and completed an in-person enrollment visit. At the enrollment visit, five individuals were excluded for reasons related to exceeding exercise criteria (N=2), pregnant (N=1), and BMI too high (N=2). This resulted in 84 individuals who were randomized to one of the four intervention arms and scheduled for baseline testing. Fourteen individuals did not return for baseline testing, for reasons related to starting medication (N=1), pregnancy (N=1), withdraw for personal reasons (N=3), and lost to follow-up (N=9); four individuals completed baseline testing, but dropped out before completing mid-or post-testing due to sickness (N=2) and lack of time (N=1), and were excluded from the final analysis. Full CONSORT information is reported in figure 1.

A final 66 overweight and obese men (N=33) and women (N=33) between the ages of 25 – 50 years completed baseline testing (Race: 69% White, 13% Black, 4% Hispanic, 3% Asian, 11% Two or more races; Age: 36.7 ± 6.0 years; Height: 171.4 ± 9.8cm; Weight: 94.5 ± 14.7 kg; %BF: 36.0 ± 7.8%) (Table 1). Overweight and obese was defined for men as a body mass index (BMI) of 28 – 40 kg/m^2 and/or body fat percentage (%BF) ≥ 25%, and for women as a BMI of 25 – 40 $kg \cdot m^{-2}$ and/or %BF ≥ 30% [21], determined by measured height (stadiometer; Perspective Enterprises, Portage, MI, USA) and weight (mechanical scale; InBody770, BioSpace, Seoul, South Korea) and %BF from bioelectrical impedance analysis (InBody770, BioSpace, Seoul, South Korea), respectively. Women were eumenorrheic, determined as reporting consistent menstruation for the three months prior to enrollment, and confirmed not-pregnant by a urine HCG pregnancy test. Participants were otherwise healthy, non-smokers, who participated in less than 150 minutes per week of moderate exercise, less than two days per week of resistance training, and had not participated in HIIT in the previous 12 weeks; participants were

instructed to maintain habitual lifestyle and activity levels for duration of the study. Individuals were excluded from participation if they: 1) had current and/or history of cardiovascular disease, diabetes, metabolic, thyroid, pulmonary, renal, hepatic, gastrointestinal, musculoskeletal disorders or any medical or surgical events, 6-months prior to enrollment; 2) had uncontrolled hypertension or an abnormal electrocardiogram; 3) were taking medications inconsistently (i.e. blood pressure medication, anti-depressants, anti-anxiety, hormonal contraceptives) or were taking a medication that could influence primary study outcomes (i.e. metformin, insulin, thyroid); 4) had lost or gained greater than eight pounds within three months prior to enrollment; 5) were consuming a high protein diet, defined as consuming $\geq$1.6 $g \cdot kg^{-1} \cdot day^{-1}$ [65]; 6) were consuming meal replacements or dietary supplements within eight weeks prior to enrollment, specifically protein, creatine, beta-alanine, carnosine, taurine, or beta-hydroxy beta-methylbutyate; or 7) had known sensitivity to the EAA supplement; 8) participated in another clinical trial that may influence study outcomes within four weeks prior to enrollment; 9) had severely impaired hearing or speech or inability to speak English; 11) were unwilling or unable to comply with the study protocol, including abstaining from food and caloric beverages (12 hrs), caffeine (12 hrs), alcohol (24 hrs), and physical activity (24 hrs) prior to testing days.

<Figure 1: CONSORT information>

Experimental Design

Using a 2:2:2:1 block randomized design, individuals were randomly assigned to one of four, eight-week intervention groups: 1) HIIT, two days per week of cycle ergometry; 2) essential amino acids (EAA) supplementation (7.2 grams EAA daily); 3) HIIT+EAA; or 4) control (CON), no intervention maintaining normal diet and exercise habits (Figure 2). Measurements of body composition, metabolic rate, substrate metabolism, and cardiorespiratory fitness were measured at baseline, 4weeks, and 8weeks; cardiometabolic markers were measured at baseline and 8-weeks. All participants provided written informed consent, completed a health history questionnaire to confirm inclusion/exclusion criteria, and

underwent a 12-lead electrocardiogram (EKG) prior to baseline testing. Participants were asked to abstain from food and caloric beverages (12 hrs), caffeine (12 hrs), alcohol (24 hrs), and physical activity (24 hrs) prior to testing sessions and removed all metal, plastics, and heavy clothing upon arrival, to avoid interference with measures. All procedures were approved by the University Biomedical Institutional Review Board.

<Figure 2: Experimental Design>

Procedures

Body Composition

A four compartment (4C) model, (Equation 1) was used to estimate fat mass (FM), percent body fat (%BF), and fat free mass (FFM) [98]. Components of this equation include: 1) body volume (BV; Equation 2), derived from a dual-energy x-ray absorptiometry total body scan (DXA; GE Lunar iDXA, GE Medical Systems Ultrasound & Primary Care Diagnostics, Madison, WI, USA) [98]; 2) total body water (TBW), measured using multi-frequency bioelectrical impedance spectroscopy (BIS; SFB7, ImpediMed, Queensland, Australia); and 3) total body bone mineral density (Mo; Equation 3), calculated using total body bone mineral content (BMC), measured from the DXA.

Equation 1: FM (kg) = 2.748(BV) – 0.699(TBW) + 1.129(Mo) – 2.051(BM)

%BF = (FM/BM) × 100

LM (kg) = BM – FM

Equation 2: $\text{BV (L)} = \frac{FM}{0.84} + \frac{LM}{1.03} + \frac{BMC}{11.63} + (-3.12)$

Equation 3: Mo = BMC × 1.0436

Test re-test reliability for the 4C model from our laboratory with a similar population is reported as intraclass correlation coefficient (ICC) of 0.995, 0.982, 0.996, standard error of measure (SEM) of

0.831 kg, 0.960%, 0.999 kg, and minimum difference (MD) of 2.30 kg, 2.6%, 2.75 kg for FM, %BF, and LM, respectively.

Dual-energy x-ray absorptiometry

For total body DXA scans, subjects were positioned in a supine position in the center of the scanning table, with arms and legs inside the scanning parameter. All DXA scans were performed and analyzed by a trained technician, following manufacturer guidelines and using manufacturer software (enCORE Software Version 16). Visceral adipose tissue (VAT) mass (kg^2) and volume (cm^3) was quantified from the pre-defined android ROI set by DXA software. This region is defined as the area spanning 20% of the distance from the top of the iliac crest to the base of the skull [99]. Test-retest reliability for VAT measurements from our lab are as follows: Mass (ICC=0.98, SEM=0.11 kg, and MD=0.22 kg) and volume (ICC=0.98, SEM=118.73 cm^3, and MD=233.85 cm^3).

Bioelectrical impedance spectroscopy

For the determination of TBW, leads were connected to four electrodes placed on the right wrist (bisecting the ulnar head), five centimeters distally on the hand, the right ankle (bisecting the malleoli), and five centimeters distally on the foot, while the participant lay supine with separation between the limbs. The average of two measurements was recorded for TBW, intracellular fluid (ICF), and extracellular fluid (ECF).

Resting Metabolic Rate and Substrate Metabolism

Resting metabolic rate (RMR) and respiratory exchange ratio (RER) were evaluated using indirect calorimetry with a ventilated canopy (TrueOne 2400, ParvoMedics, Inc., Sandy, UT). Respiratory gases, oxygen uptake, and carbon dioxide production, were analyzed over 30 second intervals for 30 minutes while lying supine in a quiet room. The percentage of carbon dioxide was maintained between 1.0 – 1.2%, with the first five minutes of the test discarded to allow for gases to normalize; RMR and

RER were averaged over the remaining 25 minutes of the test. Test-retest reliability from our lab are as follows: RMR (ICC=0.94, SEM=125.6 kcal·day^{-2}, MD=244.3 kcal·day^{-2}) and RER (ICC=0.83, SEM=0.03 arbitrary units (a.u.), MD=0.05 a.u.).

Cardiorespiratory Fitness

Peak oxygen consumption (VO_2) and ventilatory threshold (VT) were determined from a ramp based exercise test on an electronically braked cycle ergometer (Corival Lode, Gronigen, The Netherlands). Following a two minute warm-up at 20watts (W), intensity increased 1W every 3 seconds until volitional fatigue; participants were instructed to maintain a pedal cadence between 60-80 rpms for the entirety of the test. Respiratory gases were analyzed breath-by-breath using indirect calorimetry (Parvo Medics TrueMax 2400®, Salt Lake City, UT); the three highest oxygen consumption values were averaged and recorded as VO_2 (VO_{2rel}; ml/kg/min). In accordance with pre-established criteria [108], the test was considered maximal if it met a minimum of two of the following criteria: a plateau or heart rate within 10% of age-predicated HR max; a plateau or increase of no more than 150 ml/min in VO_2; or achieved an RER >1.15. Ventilatory threshold (VT) was determined as the intersection point of two linear regression lines fitted to the upper and lower portion of the ventilation versus VO_2 curve using manufacturer software (True One 2400® Metabolic Measurement System, Parvo-Medics Inc., Provo UT) [109]. Heart rate (HR) was monitored throughout the test (Polar Electro Inc., Lake Success, NY); the highest HR achieved during the test was recorded as the max HR. The highest wattage achieved during the exercise test was used to set an appropriate individualized training load for the start of the HIIT protocol. Test-retest reliability for the VO_2 protocol are as follows: ICC=0.98 and SEM=1.74 ml/kg/min.

Cardiometabolic Blood Markers

Fasting blood glucose (GLU), total cholesterol (TC), HDL-cholesterol (HDL), and non-HDL (nHDL) were measured from a whole blood sample, immediately analyzed using an Alere Cholestech

LDX® Analyzer. All blood draws were drawn and analyzed in the Applied Physiology Lab, University of North Carolina at Chapel Hill, Chapel Hill, NC.

Dietary Intake

Subjects were asked to complete three-day dietary logs at baseline, 4weeks, and 8weeks to account for the influence of normal dietary intake. Subjects were instructed to record all food and drink consumed on two, non-consecutive weekdays and one weekend day. Subjects were given detailed verbal and printed instructions on how to complete the diet logs and estimate portion sizes. Instructions included: recording the amount of food, drink, gum, candy, condiments, supplements, etc. as consumed at each meal and snack throughout the day and providing as much detail about portion size, brand names, and preparation techniques as possible. Diet logs analyzed for average calories (CAL; kcal), carbohydrate (CHO; g), fat (FAT; g), and protein (PRO; g) and relative protein (g/kg body mass) intake using nutrition analysis software (The Food Processor, version 10.12.0, Esha Research, Salem, OR, USA).

High-Intensity Interval Training

Training sessions occurred two days per week for eight weeks. All training occurred on an electronically braked cycle ergometer (Corival Lode, Gronigen, The Netherlands) with one-on-one supervision from trained research personnel. Each session consisted of a self-selected warm-up (≤5 minutes), followed by alternating sets of one minute at 90% maximal intensity (as determined from graded exercise test) and one-minute recovery at complete rest (Figure 3A). Participants were instructed to maintain a pedal cadence between 60-80 rpm; heart rate and rating of perceived exertion were recorded after each interval. Training sessions were separated by at least 24 hours, with preferential scheduling on non-consecutive days.

All participants completed six sets of intervals during week 1; an additional set was added during weeks 2-5, until reaching 10 sets. 10 sets were maintained for weeks 6 – 8 (Figure 3B). To maintain an appropriate individualized high-intensity workload, intensity (watts) was increased as a result of a ride-to-

fatigue during the last set of each training session in which participants were instructed to ride until volitional fatigue. If the individual was able to ride for an additional 15 seconds (75 seconds or longer), workload was increased by 7% at the next session (based on unpublished pilot data); if the individual rode for <75 seconds, the resistance was maintained for the next session. Completion of at least 13 sessions (80%) was considered compliant.

<Figure 3: HIIT protocol>

Essential Amino Acid Supplementation

Participants were instructed to consume an EAA powder mixed with water (8-12 oz), two times per day (REAAL, Twinlab Corporation, Hauppauge, NY, USA). One serving of the powder contained 3.6 g of a patented-ratio blend of L-leucine, L-lysine HCl, L-valine, L-isoleucine, L-arginine, L-threonine, L-phenylalanine, L-methionine, L-histidine, and L-tryptophan, formulated to support muscle growth. Participants were instructed to consume the supplement between meals; one serving between the hours of 9:00am – 12:00pm and the second serving between the hours of 3:00pm – 11:00pm, with at least 3 hours separating doses. For participants randomized to the HIIT+EAA group, the EAA supplement was consumed 30 minutes prior to- and 30 minutes after the HIIT session on training days, provided by the study staff. All participants were given a log to record supplement consumption at home. Empty tubs were returned and collected at 4weeks and 8weeks to track compliance.

Statistical Analysis:

A modified intent-to-treat analysis was conducted, including only participants who completed mid- (N=66) and/or post-testing (N=62). Group-by-time interaction effects on body composition (FM, LM, %BF, VAT), metabolic rate, substrate utilization, and cardiorespiratory fitness were evaluated using separate 4 × 2 [group (EAA vs. HIIT vs. HIIT+EAA vs. CON) × time (4week vs. 8 week)] mixed factorial ANCOVAs, covaried for baseline values. Differences in cardiometabolic markers between

groups at 8weeks were evaluated using one-way ANCOVAs [group (EAA vs. HIIT vs. HIIT+EAA vs. CON) × time (8 week)], covaried for baseline values. In the absence of a significant interaction effect, the interaction term was removed from the model to evaluate simple main effects. One-way repeated measures ANOVAs were used to evaluate simple main effects for time; one-way between-subject ANOVAs were used to evaluate simple main effects for group. Significant one-way ANOVAs were followed by pairwise t-tests using Bonferroni corrections for multiple comparisons. 95% confidence intervals (95% CI) on mean change scores adjusted for baseline values were also completed to assess changes from 0-4weeks, 4-8weeks, and 0-8weeks. If the 95% CI included zero, the mean change score was not considered statistically significant or no statistically significant change ($p>0.05$). If the 95% CI interval did not include zero, the mean change score was considered statistically significant ($p \leq 0.05$).

To explore the modulatory effects of sex on body composition and metabolic responses, Group-by-time-by-sex interaction effects on body composition, metabolic rate, substrate utilization, and cardiorespiratory fitness were evaluated using separate 4 × 2 × 2 [group (EAA vs. HIIT vs. HIIT+EAA vs. CON) × time (4week vs. 8 week) × sex (male vs. female)] mixed factorial ANCOVAs, covaried for baseline values, using the same procedures as described for full group effects. Group-by-sex differences in cardiometabolic markers at 8weeks were evaluated using 4 × 2 mixed factorial ANCOVAs [group × sex], covaried for baseline values. 95% CI were also completed to assess changes from 0-4weeks, 4-8weeks, and 0-8weeks. All statistical computations were performed using SPSS (Version 21, IBM, Armonk, NY, USA), using an $\alpha = 0.05$ to determine statistical significance.

RESULTS:

Adherence to the HIIT, EAA, and HIIT+EAA protocols was evaluated based on number of sessions completed and/or total grams of EAA supplement consumed. For the HIIT protocol, average compliance for the entire 8 weeks was 96% based on number of sessions completed; average compliance was 98% for weeks 0-4 and 95% for weeks 4-8. For EAA supplementation, average compliance for the

entire 8 weeks was 89% based on percentage of grams of supplement consumed; average compliance was 91% for weeks 0-4 and 85% for weeks 4-8.

Body composition

When controlling for baseline values, there were no significant interaction or main effects for group or time for FM, %BF, FFM, or VAT ($p>0.05$) (Table 2). Analysis of change scores with 95% CI showed no significant changes in FM, %BF, or FFM for any group at any time point ($p>0.05$). There was a small increase in VAT for HIIT+EAA from 4-8wks (Adjusted Mean Difference [Δ] ± Standard Error [SE][95%CI]: 0.07±0.03 kg; [0.004,0.14]), but no change from 0-8 weeks (Δ: -0.01±0.06 kg; [-0.11, 0.12]).

When evaluating differences between males and females, there were no significant sex interactions for FM ($p=0.685$), %BF ($p=0.749$), FFM ($p=0.843$), or VAT ($p=0.958$). Based on analysis of change scores stratified by sex, there were no significant sex-specific changes from 0-4 wks for FM, %BF, FFM, or VAT ($p>0.05$). During weeks 4-8, men in HIIT+EAA increase in FFM (Δ: 1.40±0.67 kg; [0.05, 2.74]) for men; in women, there were no significant body composition changes. During weeks 0-8, men in HIIT increased VAT (Δ: 0.15±0.06 kg; [0.04, 0.30]); there were no other significant changes in body composition for men or women.

Resting Metabolic Rate and Substrate Metabolism

When controlling for baseline values, there was no significant group-by-time interaction for RMR ($p=0.746$) or RER ($p=0.390$) (Table 3). There was no main effect for group for RMR ($p=0.695$) or RER ($p=0.489$). There was no main effect for time for RMR ($p=0.875$); based on analysis of change scores with 95% CI there was a significant increase in RMR for HIIT from 0-4 wks (Δ: 71.02±26.57 kcal/d; [18.09,123.96]) and 0-8 wks (Δ: 78.40±28.81 kcal/d; [20.71,136.08])(Figure 4); there was also a significant increase in RMR for EAA from 0-4 wks (Δ: 54.45±25.68 kcal/day; [3.10,105.81]). For RER, there was a significant main effect for time ($p=0.021$) with RER decreasing over time (V1: 0.78±0.5 a.u.;

V2: 0.77 ± 0.03 a.u.; V3: 0.75 ± 0.03 a.u.; p=0.021). Evaluating group specific changes RER decreased for HIIT from 4-8 wks (Δ: -0.04±0.01 a.u.; [-0.07,-0.02]) and 0-8 wks (Δ: -0.04±0.01 a.u.; [-0.07,-0.02]); there was also a significant decrease for EAA from 0-8 wks (Δ: -0.03±0.01 a.u.; [-0.06,-0.01]) (Figure 4).

<Figure 4: Change in RMR and RER from 0-8 weeks with 95% CI>

When evaluating differences between males and females, there was no significant sex interaction for RMR (p=0.157) or RER (p=0.734). In men, analysis of change scores with 95% CI showed a significant increase in RMR for EAA from 0-4 wks (Δ: 114.99±35.93 kcal/d; [43.04,186.94]) and 0-8 wks (Δ: 92.15±39.01 kcal/d; [13.91; 170.40]); there was also a significant increase in RMR from 0-4 wks for HIIT+EAA (Δ: 91.12±36.48 kcal/d; [18.07,164.17]) and from 0-8 wks for HIIT (Δ: 133.92±45.51 kcal/d; [42.63,225.21]). In women, analysis of change scores with 95% CI showed a significant increase in RMR for CON from 4-8 wks (Δ: 132.49±58.92 kcal/d; [14.31,250.67]); there were no other significant changes in RMR in women. For RER change scores with 95% CI showed a significant decrease in RER during 4-8 wks for HIIT in men (Δ: -0.04±0.02 a.u.; [-0.08,-0.01]) and women (Δ: -0.04±0.02 a.u., [-0.08,-0.01]). During 0-8 wks there was a significant decrease in RER for EAA in men (Δ: -0.04±0.02 a.u.; [-0.08,-0.01]) and HIIT in women (Δ: -0.05±0.02 a.u.; [-0.08,-0.02]).

Cardiorespiratory Fitness

When controlling for baseline values, there was no significant group-by-time interaction for VO_{2rel} (p=0.215), or VT (p=0.311) (Table 4). There was a significant main effect for group for VO_{2rel} (p=0.002); HIIT and HIIT+EAA had a significantly higher VO_{2rel} than CON (p=0.016, p=0.005, respectively) (AdjMean Difference [MD] ± SE: [HIIT: 3.16±1.04 ml/kg/min] [HIIT+EAA: 3.55±1.03 ml/kg/min]). There was a significant main effect for time (p=0.034) for VO_{2rel} (V1: 28.84±6.85 ml/kg/min; V2: 30.45±0.45 ml/kg/min; V3: 31.77±0.45 ml/kg/min) (Figure 5). Based on analysis of change scores with 95% CI, there was a significant increase in VO_{2rel} for HIIT from 4-8 wks (Δ±SE:

5.76±1.33 ml/kg/min; [3.11,8.42]) and 0-8 wks (Δ: 5.06 ±0.88 ml/kg/min; [3.29,6.83]). There was a significant increase in VO_{2rel} for HIIT+EAA from weeks 0-4 (Δ ± SE: 3.69±1.34 ml/kg/min; [1.34,1.02]) and 0-8 (Δ: 4.05±0.91 ml/kg/min; [1.02,6.37]); there was also in increase in VO_{2rel} from weeks 0-8 for EAA (Δ: 2.04±0.93 ml/kg/min; [-1.48,3.74]). For VT, there was not a main effect for group (p=0.642), but there was a significant main effect for time (V1: 1.39±0.37 L/min; V2: 1.40±0.40 L/min; V3: 1.54±0.40 L/min; p=0.030). Based on analysis of 95% CI, there were no significant increase in VT from 0-4 wks. There was a significant increase in VT from 4-8 wks and 0-8 wks for HIIT (4-8wks: 0.27±0.08 L/min; [0.11,0.44]; 0-8 weeks: 0.23 ±0.08 L/min; [0.08,0.38]) and HIIT+EAA (4-8 wks: 0.26±0.09 L/min; [0.09,0.43]; 0-8wks: 0.25±0.08 L/min; [0.09,0.41]).

<Figure 5: Adjusted mean relative VO_2 by group for men and women>

When evaluating differences between males and females, there was no significant sex interaction for VO_{2rel} (p=0.713), or VT (p=0.885) (Figure 5A,5B). Analysis of change scores with 95% CI showed a significant increase in VO_{2rel} from 0-4 wks for HIIT+EAA in men (Δ: 5.98±1.84 ml/kg/min; [2.30,9.66]); there were no significant changes in women. During 4-8 wks there were no significant increases in VO_{2rel} for men; in women there was a significant increase in VO_{2rel} for HIIT (Δ: 7.90±1.85 ml/kg/min; [4.20,11.61]). During 0-8 wks there was a significant increase in VO_{2rel} for HIIT (Δ: 5.85±1.27 ml/kg/min; [3.32,8.39]), EAA (Δ: 3.59±1.20 ml/kg/min; [1.18,6.00]), and HIIT+EAA (Δ: 4.96±1.32 ml/kg/min; [2.30,7.62]) in men; in women there were significant increases in VO_{2rel} for HIIT (Δ: 4.21±1.22 ml/kg/min; [1.76,6.66]) and HIIT+EAA (Δ: 3.33±1.27 ml/kg/min; [0.77,5.88]). Evaluation of 95% CI demonstrated no significant increase in VT from 0-4 wks for men or women. During 4-8 wks there was a significant in VT for HIIT+EAA in men (Δ: 0.30±0.13 L/min; [0.04,0.55]; p=0.X) and HIIT in women (Δ: 0.36±0.12 L/min; [0.12,0.60]). During 0-8 wks where was a significant increase in VT for HIIT in men (Δ: 0.28±0.11 L/min; [0.06,0.49]) and HIIT+EAA (Δ: 0.48±0.0.11 L/min; [0.26,0.71]); there were no significant changes in women.

Cardiometabolic Blood Markers

When controlling for baseline values, there was no significant differences observed between groups for TC (p=0.986), HDL (p=0.905), nHDL (p=0.988), or GLU (p=0.430). Analysis of 95% CI showed no significant changes in TC, HDL, or nHDL for any group from 0-8wks (p>0.05). There was a small increase in GLU for HIIT+EAA from 0-8 wks (HIIT+EAA (4.45±2.19 mg/dL; [0.06,8.84]) (Table 5).

When evaluating differences between males and females, there was no significant sex interaction for TC (p=0.939), HDL (p=0.498), nHDL (p=0.773), or GLU (p=0.945). Analysis of 95% CI showed no significant changes in any cardiometabolic markers in men or women at any time point.

Dietary Intake

When controlling for baseline values, there was no significant interaction effect for calories (p=0.618), CHO (p=0.492), PRO (p=0.831), FAT (p=0.634), or relative PRO (p=0.891); there were also no main effects for group or time (p>0.05) (Table 6). Based on analysis of 95% CI, there were no significant changes in CAL or FAT at any timepoint. For CHO, there was no change from 0-4 wks, a small decrease from 4-8 wks for EAA (Δ: -36.29 ± 16.30 g/d; [-69.01,-3.58]), but no changes from 0-8 wks. For PRO, there was a small increase from 0-4 wks for EAA (Δ: 14.99 ± 5.99 g/d; [2.99,26.98]); there were no changes from 4-8 wks or 0-8 wks. There was a similar trend with relative PRO, with a small increase from 0-4 wks for EAA (Δ: 0.156 ± 0.06 g/kg/d; [0.03,0.28]), but no changes from 4-8 wks or 0-8 wks. When evaluating differences between males and females, there was no significant sex interaction effect for calories (p=0.908), CHO (p=0.972), PRO (p=0.744), FAT (p=0.848), or relative PRO (p=0.579).

DISCUSSION:

Despite the high-intensity nature, HIIT has been shown to be a feasible, effective, and enjoyable form of exercise to increase cardiorespiratory fitness and improve cardiometabolic health in overweight and obese individuals [11, 113, 118]. Decreased body fat and increases in LM have also been reported with HIIT [14, 15, 34, 48, 51, 80, 114, 115]. To date, few studies have evaluated the combined effects of a minimal nutritional intervention, such as EAA, with HIIT on body composition and metabolic characteristics. Results of the current study showed minimal effects of eight weeks of HIIT, with or without EAA, on body composition. HIIT and EAA supplementation separately promoted increases in metabolic rate (HIIT: +78.40 kcal/d) and fat oxidation (HIIT: +13%; EAA: +10%) after 8-weeks. Consistent with previous research, HIIT is an effective form of exercise for improving cardiorespiratory fitness, with an average increase in VO_2 of 1.56 ml/kg/min and 4.56 ml/kg/min after 4- and 8-wks, respectively. EAA supplementation combined with HIIT did not provide any additional benefit. Consistent with previous research [35, 81-88], no modulatory effect of sex was observed, suggesting that HIIT may overcome genetic differences in exercise response, resulting in similar body composition and metabolic benefits for men and women [78].

Body Composition

Previous results of four different meta-analyses suggest that HIIT elicits similar reductions in FM and %BF as traditional moderate intensity exercise [48, 51, 113, 115], but requires significantly less overall training time and volume (20 min, 2-3 d/wk vs. >30min 5-6 d/wk). This makes HIIT a potentially appealing approach for achieving fat loss and cardiometabolic benefits. In the current study 8-weeks of HIIT and/or EAA supplementation had no effect on FM (Δ: -0.1 – +0.4 kg) or %BF (Δ: -0.03 – +0.3%); there was a small increase in VAT with HIIT+EAA from 4-8wks (+0.07 kg) that did not exceed error of the measure. It has previously been suggested that fat loss with HIIT is favored in longer interventions, with an expected FM loss of about 2 kg in about 10 weeks [48] or about 1.6 kg in 8 weeks. Although changes in the current study did not reach this magnitude, previous studies that are closer in duration to

the present one, have reported -0.6 kg and -0.9 kg decreases in FM after 6- and 8-weeks of HIIT in overweight/obese adults [34, 52]. In contrast, a loss of 1.96 kg FM following three weeks of HIIT was reported in overweight and obese women [13]. In all of these previous studies, HIIT sessions were conducted 3 days/week, compared to the 2 days/week in the current study. Two days/week was selected for feasibility and compliance purposes, but more frequent training sessions may influence the amount of fat loss achieved with HIIT. In addition to total body fat, there is evidence suggesting that high-intensity exercise is especially effective for reducing VAT [119]. No significant changes in VAT were observed in the current study (Δ: -0.01 - +0.05 kg). Lack of change in the current study may be related to low average levels of VAT in the current cohort, as well as method of measurement [119, 120]. Regardless, fat loss with exercise often does not become pronounced until combined with a larger nutritional intervention, such as caloric restriction [62, 63]. The present study was not aimed at reducing calories, but the provision of additional EAAs, which amounts to little calories. No significant changes in caloric intake were observed in the current study (Table 5). Therefore, HIIT may promote initial decreases in FM, but the effect of HIIT alone, with or without the addition of the EAA supplement, is likely not enough to offset the impact of normal dietary intake.

Recent data has shown HIIT may also support increases in FFM [14, 15, 34, 48, 51, 80, 114, 115]. Significant increases in FFM (1.2 kg and 0.6 kg) have previously been reported after 12- and 6-weeks of sprint intervals in overweight men and recreationally active college students, respectively [54, 80]. Although there were no significant changes in FFM for the full group in the current study (Δ: -0.21 – +0.18 kg), there was a significant 1.4 kg increase in FFM for men in the HIIT+EAA group from 4-8 wks, and a non-significant 1.0 kg increase from 0-8 wks, based on 95% CI, similar to the changes reported in previous studies. These results could be indicative of EAA supplementation supporting increases in FFM with HIIT. It should be noted however, that a non-significant, but similar change was observed in CON (4-8wks: +2.0 kg; 0-8wks: +1.0 kg), making the implications of the change observed with HIIT+EAA difficult to interpret. Lack of significant change in FFM may be related to low relative dietary protein intake. Average relative protein intake in the current study was 0.95 g/kg/d, well below recommendations

for optimizing health (1.2-1.6 g/kg/d) [121]. Even with the addition of EAA, it is likely that individuals were not consuming enough protein to support large changes in FFM. Lack of significant changes in FFM may also be related to measurement of total body FFM, which may not be sensitive to the cycling modality of HIIT used in the current study, which almost exclusively targets the legs. Previous studies utilizing cycle ergometry have reported significant increases in leg FFM (0.2-0.3 kg), despite non-significant increases in total body FFM after 3- (1.9 kg, 2.2 kg) and 6-weeks (0.6 kg) of HIIT [13-15, 34]. Although the current study utilized a gold-standard 4-compartment model for estimation of body composition, a majority of studies utilize DXA for quantification of FFM. DXA has been shown to overestimate LM in overweight and obese individuals, due to the influence of adipose FFM [122, 123]. Quantification of regional changes in FFM, specifically in the legs, utilizing other methods for estimating muscle changes, such as ultrasound, might prove to be more sensitive to potential adaptations to HIIT and EAA supplementation.

Metabolism

Fat loss associated with HIIT is thought to be a result of increased post-exercise energy expenditure and enhanced fat oxidation associated with increased mitochondrial biogenesis and oxidative capacity [12, 16, 17]. Despite non-significant changes in body composition in the current study, significant increases in energy expenditure (+78.40 kcal/d) and fat oxidation (RER: -0.04 a.u) were observed with HIIT across the entire 8-weeks. Interestingly, significant changes in energy expenditure occurred predominately from weeks 0-4 (+71.02 kcals/d), while significant changes in fat oxidation occurred predominately from weeks 4-8 (-0.04 a.u.). In untrained individuals, exercise has been shown to stimulate increases in both mitochondrial biogenesis and myofibrillar protein synthesis, regardless of exercise modality (i.e. aerobic vs. resistance) [124]. As training progresses, muscular adaptation becomes more specific to the exercise modality [124]. In the case of HIIT training, previous studies have shown mitochondrial adaptations to occur relatively quickly, with increases in mitochondrial proteins reported in as few as three sessions of HIIT [125]. High-intensity exercise has also been shown to stimulate

significant increases in myofibrillar protein synthesis for 24 and 48 hours post exercise [18, 55]. Applying this to the current study, increased metabolic rate could be related to early increases in mitochondrial and myofibrillar protein synthesis, combined with increased energy demands during and after a HIIT session [17]. As the training progressed into the second four weeks, it is likely that mitochondrial adaptations continued, resulting in increased oxidative capacity and subsequent decrease in RER. Further research evaluating neuromuscular adaptations to HIIT is needed to support this theory.

Results of the current study also suggest that EAA supplementation may promote metabolic changes, independent of exercise. Results showed an increase in RMR (+54.45 kcal/d) from weeks 0-4 with EAA supplementation, with a further increase from 0-8 wks in men only (+92.15 kcal/d). Fat oxidation also increased with EAA supplementation across the entire 8-weeks for the full group (RER: -0.031 a.u.). Skeletal muscle metabolism accounts for around 20-30% of RMR, the largest component of which is protein turnover, which is predominately supported by fat oxidation [60, 126]. The rise in energy expenditure and fat oxidation from EAA in the current study likely reflects an increase in protein turnover [127]. Infusion of EAA has been shown to equally stimulate both myofibrillar and mitochondrial protein at rest [128]. Therefore, EAA supplementation could be expected to support mitochondrial adaptation, albeit at a much slower rate and to a lesser extent than with exercise, ultimately leading to greater fat oxidation.

Cardiorespiratory Fitness

HIIT is a potent stimulus for improving cardiorespiratory fitness [12, 113]. Previous studies in overweight and obese adults have reported improvements in VO_2 in as little as three weeks (3d/wk; +3.4 ml/kg/min) of HIIT training [15]. Results of a meta-analysis also suggest HIIT may be more effective for increasing cardiorespiratory fitness in overweight/obese adults than moderate intensity exercise [113]. Results of the current study are consistent with previous research. Both HIIT (+5.1±0.9 ml/kg/min) and HIIT+EAA (+4.1±0.9 ml/kg/min) effectively improved VO_2 across the entire eight-week intervention, with greater increases during the first four weeks for HIIT+EAA (3.7±1.3 ml/kg/min) and greater

increases during the second four weeks for HIIT (5.8±1.3ml/kg/min). Interestingly, VO_2 also significantly increased with EAA only over the course of the intervention. Taken in context with the metabolic adaptations (RMR and RER) observed with HIIT and EAA during the first four weeks, these results could provide further evidence of a potential benefit of EAA supplementation for supporting mitochondrial adaptation. Previous studies evaluating effect of protein supplementation of aerobic capacity are limited and show mixed results [129, 130]. Knuiman et al (2019) showed twice daily protein supplementation, in combination with 10wks of endurance training, resulted in greater increases in VO_2 than endurance training alone. Interestingly, these changes were attributed to increases in LM, particularly leg LM, in addition to non-significant improvements in skeletal muscle oxidative capacity [130]. Ventilatory threshold also increased for both HIIT (+0.23 L/min) and HIIT+EAA (+0.25 L/min) across the entire eight-week intervention. In contrast to the trends observed with VO_2, greater increases in VT did not occur until the second four weeks for both groups. Similar trends have previously been reported following six-weeks of HIIT, with and without β-alanine supplementation in recreationally active college-aged men [131]. Although β-alanine and EAA impact adaption through different mechanisms, this uncoupling between adaptation in VO_2 and VT may be a reflection of different mechanisms involved with improvement for each [132]. Regardless, HIIT was effective for improving VT, with no additional benefit from EAA. Further research is needed to understand the impact of protein/EAA supplementation on cardiorespiratory adaptation.

Cardiometabolic Markers

Results of the current study showed no significant improvements in fasting blood glucose or cholesterol for any group. In contrast, there was a significant increase in fasting glucose with HIIT+EAA (Baseline: 90.58 ± 12.53; 8week: 95.22 ± 15.36), although the average was still within normal ranges (<100 mg/dL). HIIT has previously shown promise for improving cardiometabolic health markers, particularly fasting blood glucose [12, 39, 133]. In type 2 diabetics, 24-hour blood glucose concentrations were reduced following 2 weeks (3d/wk) of HIIT [134]. Similarly, in overweight and obese men, fasting

glucose and insulin sensitivity were improved after 3 weeks (3 d/wk) of HIIT [15]. There has been some controversy as to whether protein/EAA intake is detrimental to insulin sensitivity in humans. Cross-sectional analyses in overweight and obese adults show a strong correlation between protein intake and type 2 diabetes [135, 136]. In contrast, intervention trials have shown no detrimental effects in humans, and instead has been shown to stabilize postprandial and fasting blood glucose in overweight and obese women [4, 64], while having no effect on hepatic or peripheral insulin resistance or lipids older adults with metabolic syndrome [137]. High-intensity exercise has been shown to elevate blood glucose post-exercise [138-140], effects of which have been shown to last for up to 120 minutes in type 1 diabetics [138]. Rise in blood glucose is attributed to increases in catecholamines, growth hormone, and cortisol with HIIT [17, 138], but further research is needed to understand if the response observed in the current study is a positive or negative adaptation.

Lack of changes in the current study may be related to the relatively healthy nature of the participants; averages for TC, HDL, and GLU were all within normal ranges at the start of the study. Previous studies have suggested individuals with worse fasting values, were more likely to see bigger benefits, compared to those who were closer to normal ranges [39]. Longer duration interventions may also be necessary to see improvements in cardiometabolic markers [141]. In contrast to the rapid changes in cardiorespiratory fitness, it has been suggested that a minimum of eight weeks to see improvements in HDL, while significant improvements in total cholesterol, LDL, and triglycerides often do not improve with HIIT alone [133, 141].

Sex Differences

An exploratory aim of this study was to evaluate the modulatory effects of sex on the body composition and metabolic response to HIIT and EAA. Results of the current study showed similar effects of HIIT in both men and women. Sex differences in response to exercise are primarily attributed to the influence of sex hormones; estrogen has been shown to be associated with greater capacity for aerobic metabolism and fat oxidation [89], while testosterone is supportive of muscular growth and adaptation

[142]. Related to these differences, men often show greater body composition changes in response to exercise, compared to women, who in some cases have been shown to have paradoxical responses to exercise (i.e. gaining body fat) [77]. In mice, HIIT was shown to overcome this paradoxical response, specifically in females, suggesting that HIIT may overcome hormonal and genetic differences between men and women [78]. Results of the current study are in line with these findings, showing minimal differences in response to HIIT between men and women. Results do potentially indicate a difference in the rate of adaptation between men and women; significant changes in men tended to occur during the first four weeks, while significant changes in women occurred almost exclusively in the second four weeks. This trend was most prominent with changes in VO_2; significant increase in VO_2 with HIIT+EAA during the first four weeks was predominately observed in men, while the significant increase in VO_2 with HIIT that occurred during the second four weeks was predominately observed in women. Increases in RMR for men with EAA and HIIT+EAA also occurred during the first four weeks, while increases in fat oxidation for women with HIIT occurred during the second four weeks. These results could also indicate a unique effect of EAA in men, stimulating faster adaptation, to HIIT. It is unclear why EAA would differentially impact men and women, as benefits have been observed in both [116, 143], but further research in this area may provide insightful information for enhancing adaptation to exercise in men and women.

No Differential Effect of HIIT+EAA

Based on adaptations observed separately with HIIT and EAA supplementation in the current study, in combination with previous research, EAA would be expected to support muscular and mitochondrial adaptations associated with HIIT by supporting muscle recovery, protein kinetics, and mitochondrial biogenesis [144]. Nutrient timing strategies were utilized in the current study with EAA consumed 30 minutes prior to- and within 30 minutes after HIIT in order to maximize potential for adaptation. However, EAA supplementation did not appear to provide any additional benefit beyond the adaptation observed with HIIT. It is unclear why EAA supplementation in combination with HIIT did not

result in greater increases in metabolic adaptation. One theory is that the amount of EAA was not sufficient to overcome demands of HIIT, or that total overall PRO intake (average 0.95 g/kg/d) was still insufficient to support meaningful adaptations. The specific EAA supplement used in the current study was a clinically developed patented blend [116, 127]; EAA dosing was based on manufacturer recommended intakes, mimicking a realistic dosing strategy. Research concerning the protein needs and effects on HIIT adaptations, beyond a single session, are very limited, but protein needs are known to be increased with endurance training (1.2-2.4 g/kg/d) [9]. Ten-weeks of branched chain amino acid supplementation has shown to increase Wingate peak power and potentially increase time trial performance in male cyclists [145]. However, 6-10 weeks of protein supplementation in combination with endurance training failed to lead to improvements in in VO_2, performance time, and markers of mitochondrial biogenesis [144]. Another theory is that metabolic adaptation is more sensitive to carbohydrate availability, than protein. Studies in elite athletes have shown that training in a carbohydrate restricted/glycogen depleted state, withholding CHO during exercise, and/or delaying CHO intake/glycogen resynthesis after exercise, significantly enhances cell signaling pathways and upregulates oxidative enzymes that lead to increased total body and intramuscular lipid oxidation [71, 72]. While CHO consumption in these studies was shown to blunt the stimulation of key signaling pathways associated with mitochondrial adaptation, PRO consumption had no effects, and it was recommended that 20-25g of protein be consumed before, during, and/or after exercise in order to maintain protein balance and support muscular recovery [71, 73]. Therefore, the lack of consumption of carbohydrate around the exercise session, or the replacement of carbohydrate with protein throughout the day may have been the driving factor in the adaptation observed as opposed to the increase in EAA.

Conclusions

In conclusion, HIIT is an effective and feasible approach to increasing cardiorespiratory fitness and promoting positive metabolic adaptation in overweight and obese adults. Although HIIT had minimal impact on body composition, improvements in total body metabolism, significantly increasing

resting metabolic rate, fat oxidation, and cardiorespiratory fitness did occur over the course of eight weeks. Twice daily EAA consumption, between meals or before and after exercise sessions, may also stimulate positive metabolic adaptations, increasing metabolic rate and fat oxidation. These adaptations seem to be independent of exercise and did not seem to enhance/overcome adaptations observed with HIIT. Metabolic and cardiorespiratory benefits with HIIT extended to both men and women, with potential unique benefits of EAA supplementation in men, but further research is needed to understand how protein/EAA contributes to the unique adaptations of HIIT.

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CHAPTER V

MANUSCRIPT 2

HIGH-INTENSITY INTERVAL TRAINING AND ESSENTIAL AMINO ACID SUPPLEMENTATION: MUSCLE CHARACTERISTICS & MUSCLE PROTEIN TURNOVER

INTRODUCTION

The importance of lean body mass in the context of exercise performance, strength, and functionality are well recognized, but in the context of weight loss and metabolic health, benefits of maintaining high-quality lean mass (LM) are less commonly considered [60]. Relative decreases in LM begin to occur at around the age of 30, with noticeable decreases occurring at around 45-50 years [146]. A loss of LM is associated with decreases in energy expenditure, reduced function and strength, and an increased risk of weight regain [60, 147]. Rate of age-related loss in LM has been shown to be greater in the lower body/legs [146, 148]. Greater leg LM has previously been shown to be associated with higher resting metabolic rate in both men and women [149]. A decline in muscle quality, often seen with age and obesity, is also of metabolic importance. Reduced muscle quality, represented by an increase in intramuscular fat accumulation and connective tissue, has been associated with impaired insulin sensitivity [60, 150]. Effective strategies that support and promote maintenance of skeletal muscle size and quality may have an important impact on metabolic disease [60].

High-intensity interval training (HIIT), defined as bouts of vigorous exercise interspersed with periods of low-intensity exercise or rest, is an efficient form of exercise known for its rapid improvements in cardiorespiratory fitness [11, 12, 41]. Due to the significant effects of interval training on mitochondrial and cardiorespiratory changes, research has focused primarily on weight and fat loss with HIIT. However, a few recent studies have reported increases in LM and muscle size after as few as three weeks of interval

training [13-15, 34, 80, 114]. Preliminary studies from our lab have shown an average 1.9 kg and 2.2 kg increase in total body LM in overweight and obese men and women, respectively, after three weeks of HIIT [13, 15]. Although these increases in total body LM were non-significant, follow-up analysis showed a significant increase in muscle cross sectional area (mCSA) of the vastus lateralis [14]. Other studies have reported significant increases in leg LM following 6- and 12-weeks of HIIT in both overweight men and women, despite non-significant or low-magnitude changes in total body LM [34, 114]. Changes in regional assessment of muscle characteristics may be a more descriptive and sensitive measures of muscular change associated with HIIT.

Ultrasonography has grown in popularity as a non-invasive approach to evaluating muscle characteristics. Unlike most body composition devices that estimate total and regional LM, ultrasound allows for assessment of individual muscles. In addition to quantification of mCSA, ultrasonography can also be used to evaluate muscle quality and architectural features, such as fascicle length and pennation angle, which have been shown to influence muscular strength and force production [101, 151, 152]. Measures of muscle characteristics provide insight into muscular health and functionality, beyond muscle size, which to date, has not been evaluated in response to HIIT.

To stimulate an increase in muscle size, muscle protein synthesis (MPS) must exceed muscle protein breakdown (MPB) [66]. Exercise is known to stimulate MPS, with a concomitant increase in MPB, often resulting in an imbalance in protein turnover, and thus a loss in LM. [19, 66]. Protein, specifically essential amino acids (EAA), are required to increase LM [19, 66]. When EAAs are consumed prior to or following exercise, MPS is augmented, resulting in an increase in muscle size [19, 66, 68-70, 116, 153]. Few studies have evaluated the effects of HIIT in combination with a nutritional intervention [34], with none to our knowledge evaluating the effect of HIIT+EAA on LM and muscle characteristics. Improvements in LM with a minimal nutrition and exercise intervention such as this, could have significant implications for improving health outcomes and maintaining muscle quality in a variety of populations. Addition of EAA could further enhance this process by providing required amino acids to support muscular health and growth. Therefore, the purpose of this study was to compare the

independent and combined effects of HIIT and EAA supplementation total body and regional LM of the thigh, muscle characteristics, and whole-body protein turnover in overweight and obese men and women. Muscle characteristics include: cross sectional area of the superficial quadricep muscles (rectus femoris, vastus medialis, vastus lateralis), in addition to muscle quality, volume, and architectural characteristics of the vastus lateralis. An exploratory aim was to evaluate the potential modulatory effects of sex. It was hypothesized that HIIT would result in significant increases in thigh LM, specifically increasing mCSA of the superficial quadriceps muscles, in addition to improving muscle quality; no changes in architectural characteristics of the vastus lateralis were predicted. It was also hypothesized that the addition of EAA would result in an increase in whole-body protein balance, supporting greater increases in LM, mCSA, and improvements in muscle quality. Finally, it was hypothesized that improvements would occur in both men and women, with greater changes occurring in men [15, 35].

METHODS:

Participants:

Sixty-six overweight and obese men (N=33) and women (N=33) between the ages of 25 – 50 years volunteered to participate (Race: 69% White, 13% Black, 4% Hispanic, 3% Asian, 11% Two or more races; Age: 36.7 ± 6.0 years; Height: 171.4 ± 9.8 cm; Weight: 94.5 ± 14.7 kg; %BF: 38.8 ± 7.2%). For men, overweight/obese was defined as a body mass index (BMI) of 28 – 40 kg/m^2 and/or body fat percentage (%BF) ≥ 25% and for women as a BMI of 25 – 40 $kg \cdot m^{-2}$ and/or %BF ≥ 30% [21]. BMI was determined by measured height (stadiometer; Perspective Enterprises, Portage, MI, USA) and weight (mechanical scale; InBody770, BioSpace, Seoul, South Korea) and %BF from bioelectrical impedance analysis (InBody770, BioSpace, Seoul, South Korea), respectively. Women were eumenorrheic, reporting consistent menstruation for the three months prior to enrollment, and confirmed not-pregnant by a urine pregnancy test. Participants were otherwise healthy (no cardiovascular, metabolic, or surgical events within six months of enrollment), non-smokers, participating in less than 150 minutes per week of moderate exercise, less than two days per week of resistance training, and had not participated in HIIT

within 12 weeks prior to enrollment; participants were instructed to maintain habitual lifestyle and activity levels for duration of the study. Detailed descriptions of CONSORT, inclusion/exclusion criteria, and participant characteristics have previously been reported (Hirsch et al. 2020).

Experimental Design:

Individuals were randomly assigned, using 2:2:2:1 block randomization, to either 1) HIIT training, two days per week of cycle ergometry; 2) EAA supplementation, consuming 3.6 grams EAA twice daily; 3) HIIT+EAA; or 4) control (CON), maintaining normal diet and exercise habits. Measurements of body composition and muscle size, quality, and architectural characteristics were measured at baseline, 4weeks, and 8weeks. Total body protein turnover, was measured in a subsample of individuals from the HIIT (N=8), EAA (N=7), and HIIT+EAA (N=7) groups at baseline and 8weeks. All participants provided written informed consent, completed a health history questionnaire to confirm inclusion/exclusion criteria, and underwent a 12-lead electrocardiogram (EKG) prior to baseline testing. Participants were asked to abstain from food and caloric beverages (12hrs), caffeine (12hrs), alcohol (24hrs), and physical activity (24hrs) prior to testing sessions. Participants were also asked to remove all metal, plastics, and heavy clothing upon arrival, to avoid interference with measures. All procedures were approved by the University Biomedical Institutional Review Board.

Procedures:

Dual-energy x-ray absorptiometry scan

Body composition, specifically total body LM and lean mass of the thigh (thighLM), were determined from a total body dual-energy x-ray absorptiometry scan (DXA; GE Lunar iDXA, GE Medical Systems Ultrasound & Primary Care Diagnostics, Madison, WI, USA). Prior to scanning, subjects were asked to remove all metal, plastics, and heavy clothing, wearing only lightweight athletic clothing. Subjects were positioned in a supine position in the center of the scanning table, with arms and legs inside the scanning parameter. All DXA scans were performed and analyzed by a trained technician,

following manufacturer guidelines using manufacturer software (enCORE Software Version 16). For sub-analysis of thighLM, a region-of-interest (ROI) was manually drawn around the right thigh, such that, 1) the thigh was separated from trunk by a line bisecting the femoral head and touching the ischial tuberosity, as would be drawn to form the pelvic triangle; and 2) the thigh was separated from the lower shank by a line drawn bisecting the intercondylar space between the femur and the tibia (Figure 6). Test-retest reliability for DXA measurements of LM are as follows: ICC=0.998, SEM=0.806 kg, and MD=1.580 kg. Test-retest reliability for thighLM are $ICC_{2,1}$=0.999, SEM=0.196.

Ultrasound

Muscle cross-sectional area (mCSA) of the vastus lateralis (VL), rectus femoris (RF), and vastus medialis (VM) was determined from panoramic ultrasound (US) scans of the thigh (GE LOGIQ-e, Software version R8.0.7, GE Healthcare, Wisconsin, USA) using a linear array US transducer probe (GE: 12L-RS) and standardized frequency (10 Hz) and gain (50) settings [22, 23]. Measurements were made by applying the device probe directly against the skin at the peak anatomical cross-sectional area of each muscle, defined as 30%, 50%, and 60% of femur length for the VM, VL, and RF, respectively [27, 100].

Muscle volume (mV) was evaluated from cross-sectional scans of the VL taken at 25%, 50%, and 75% of muscle length [mV = (25% muscle length (cm) × 25%mCSA (cm^2)) + (25% muscle length (cm) × 50%mCSA (cm^2)) + (25% muscle length (cm) × 75%mCSA (cm^2))] [27, 100]; pennation angle (PA) and fascicle length (FL) of the VL were evaluated from panoramic scans along the fascicle plane at 50% of femur length [26]. The scans were performed by the same technician while the subject lay supine with the right leg extended and relaxed on the examination table for approximately 5 minutes.

All images were exported and analyzed using Image-J software (National Institutes of Health, USA, version 1.51). Muscle cross-sectional area was determined by tracing the outline of the muscle along the inside fascial border [22, 23]. Echo intensity (EI), was determined using grayscale analysis, with a correction for subcutaneous fat thickness [$EI = EI_{raw} + (SAT \times 40.5278)$] from the cross sectional image of the VL taken at 50% of femur length [22, 23, 101]. Fascicle length was determined as the length

of one fascicle between the superficial and deep aponeuroses, measured near the center of the image [26]; PA was determined by measuring the angle between the deep aponeurosis and the same fascicle used to determine FL [26]. The same technician performed all analysis for each outcome. Each image was individually calibrated for analysis by measuring the number of pixels in a known distance (image depth). Two images from each location were analyzed and an average of the two measures was reported for all outcomes (CSA, EI, FL, PA). Test-retest reliability for mCSA and EI from our lab are as follows: mCSA ICC=0.99, SEM of 0.744 cm^2; EI ICC=0.99, SEM=1.5 a.u.

Total Body Protein Turnover

Total body protein turnover (g N/24hr) was determined by [^{15}N]alanine isotope tracer (98% enriched, Cambridge Isotope Lab, Andover, MA) [102] in which participants ingested a 2.00 gram dose of [^{15}N]alanine mixed with water. For the 24hrs following ingestion, participants were asked to collect urine from all voids and keep a diet record of all food and drink consumed. Diet records were analyzed for protein intake (g) to account for dietary nitrogen intake. A zero and 24-hour blood draw was collected to measure blood urea nitrogen. Isotopically labeled nitrogen from the urine samples was used to determine nitrogen flux according to Fern et al. [103]. Total body protein synthesis (PS) and breakdown (PB) was calculated from urine samples according to Stein et al. [104] and used to determine net protein balance and flux. Samples were shipped and analyzed at the Center for Translational Research in Aging and Longevity, University of Arkansas Medical Sciences, Little Rock, AR. This method of analysis is considered a valid and reliable approach for analysis of total body protein turnover [154].

Dietary Intake

Three-day dietary logs were collected at baseline, 4weeks, and 8weeks to account for the influence of normal dietary intake. Subjects were instructed to record all food and drink consumed on two, non-consecutive weekdays and one weekend day. Detailed verbal and printed instructions were provided, instructing on how to complete the diet logs and estimate portion sizes. Diet logs were analyzed

for average calories (CAL; kcal), carbohydrate (CHO; g), fat (FAT; g), protein (PRO; g) and relative protein (g/kg body mass) intake using nutrition analysis software (The Food Processor, version 10.12.0, Esha Research, Salem, OR, USA). Full dietary intake data has been presented elsewhere (Hirsch et al. (2020)).

High-Intensity Interval Training

All training occurred on a cycle ergometer, two days per week for eight weeks, with one-on-one supervision. Each session consisted of a self-selected warm-up (≤5 minutes), followed by alternating sets of one minute at 90% max wattage and one-minute recovery at complete rest. Training started with six sets of intervals and progressed by one set each week until reaching 10 sets at week five; 10 sets were maintained for the remainder of the 8 weeks (Figure 3). To maintain an appropriate individualized high-intensity workload, individuals were asked to ride to fatigue, on the last set of each session. If the individual was able to ride for >75 seconds, resistance was increased by 7% at the next session; if the individual rode for less than ≤75 seconds, resistance was maintained for the next session (based on unpublished pilot data) (Figure 2). Training sessions were separated by at least 24 hours, with preferential scheduling on non-consecutive days. Starting intensity was individualized for each participant based on maximum wattage reached during baseline cardiovascular fitness (VO_2peak) testing (previously described in manuscript 1 or reference another study). Adherence for the entire 8 weeks was 96% based on number of sessions completed; average compliance was 98% for weeks 0-4 and 95% for weeks 4-8.

Essential Amino Acid Supplementation

The EAA supplement, formulated to support muscle growth, contained 3.6 g of a patented-ratio blend of L-leucine, L-lysine HCl, L-valine, L-isoleucine, L-arginine, L-threonine, L-phenylalanine, L-methionine, L-histidine, and L-tryptophan (REAAL, Twinlab Corporation, Hauppauge, NY, USA). Participants were instructed to consume the EAA powder mixed with water (8-12 oz), two times per day between meals; one serving between the hours of 9:00am – 12:00pm and the second serving between the

hours of 3:00pm – 11:00pm, with at least 3 hours in between doses. On training days, participants assigned to the HIIT+EAA group consumed one serving 30 minutes prior and following exercise. Subjects were asked to record the time of day the supplement was taken on a supplement log. Supplement containers were also collected and weighed at 4weeks and 8weeks to track compliance. Average compliance for the entire 8 weeks was 89% based on percentage of grams of supplement consumed; average compliance was 91% for weeks 0-4 and 85% for weeks 4-8.

Statistical Analysis:

A modified intent-to-treat analysis was conducted, including only participants who completed mid- (N=66) and/or post-testing (N=62). Group-by-time interaction effects on total body LM and thighLM, were evaluated using separate 4 × 2 [group (EAA vs. HIIT vs. HIIT+EAA vs. CON) × time (4week vs. 8 week)] mixed factorial ANCOVA's, covaried for baseline values. Secondary outcomes including muscle size (mCSA, MV), quality (EI), and architecture characteristics (FL, PA) were also evaluated using 4 × 2 mixed factorial ANCOVA's, covaried for baseline values. Differences in whole-body protein turnover between groups (HIIT vs. EAA vs. HIIT+EAA) at 8weeks were evaluated using one-way ANCOVAs, covaried for baseline values. For significant interactions, one-way repeated measures ANOVA's were used to evaluate simple main effects for time; one-way between-subject ANOVA's were used to evaluate simple main effects for group. Significant one-way ANOVA's were followed by pairwise t-tests using Bonferroni corrections for multiple comparisons. Mean change scores adjusted for baseline values with 95% confidence intervals (CI) were also completed to assess changes from 0-4weeks, 4-8weeks, and 0-8weeks. If the 95% CI included zero, the mean change score was not considered statistically significant ($p>0.05$). If the 95% CI interval did not include zero, the mean change score was considered statistically significant ($p \leq 0.05$).

Group by time by sex interaction effects on total body LM, thighLM, and muscle size, quality, and architecture characteristics were evaluated using separate 4 × 2 × 2 (group × time × sex) mixed factorial ANCOVA's, covaried for baseline values, using the same procedures as described above. All

statistical computations were performed using SPSS (Version 21, IBM, Armonk, NY, USA), using an $\alpha = 0.05$ to determine statistical significance.

RESULTS:

Total Body and Thigh Lean Mass

Average total body and thigh LM values are presented in table 7. When adjusting for baseline values, there was no significant group-by-time interaction (p=0.654) or main effect for group (p=0.771) or time (p=0.076) for total body LM. Based on analysis of mean change scores with 95% CI, there were no changes in total body LM for any group from weeks 0-4 and 4-8; from weeks 0-8 there was a small increase in total body LM for HIIT+EAA (adjusted mean change (Δ) ± SE [95% CI]: 0.66 ± 0.27 kg; [0.11, 1.20]), but no other group.

For thighLM, there was no interaction (p=0.636) or main effect for time (p=0.176), but there was a main effect for group (p=0.003); HIIT (adjusted Mean±SE: 7.29 ± 0.04 kg; p=0.035) and HIIT+EAA (7.34 ± 0.04 kg; p=0.003) had significantly greater thighLM than CON (7.14 ± 0.05 kg). Based on mean change scores (Figure 7), thighLM increased from weeks 0-4 for HIIT (Δ: 0.09 ± 0.04kg; [0.01,0.16]), EAA (Δ: 0.07±0.04 kg; [0.001,0.15]), and HIIT+EAA (Δ: 0.13 ± 0.04 kg; [0.06,0.20]). From weeks 4-8 there were further increases for HIIT (Δ: 0.09 ± 0.04 kg; [0.04,0.01]) and HIIT+EAA (Δ: 0.09 ± 0.04 kg; [0.01,0.17]), resulting in significant increases from weeks 0-8 for both HIIT (Δ: 0.17 ± 0.05; [0.08, 0.27]) and HIIT+EAA (Δ: 0.22 ± 0.05; [0.12,0.31]). There were no changes for CON. There was no significant difference in change between groups (p>0.05).

Muscle Cross Sectional Area

Average mCSA for the RF, VM, and VL are presented in table 8. There was no significant interaction effect for mCSA of the RF (p=0.976), VM (p=0.781), or VL (p=0.477). For the RF, there was no main effect for group (p=0.284) or time (p=0.836) and mean change scores showed no significant changes in mCSA from weeks 0-4, 4-8, or 0-8. For VM mCSA, there was a main effect for group

(p=0.044), but not time (p=0.952). Post hoc analysis showed no significant differences between groups for the VM (p>0.05); analysis of change scores showed no significant changes from weeks 0-4 or 4-8, but an increase in VM mCSA for CON from weeks 0-8 (Δ: 1.59 ± 0.79 cm^2; [0.01,3.18]). For the VL, there was a significant main effect for group (p<0.001) and time (p=0.041). VL mCSA was significantly greater in HIIT (adjusted mean±SE: 27.08 ± 1.21 cm^2) than EAA (25.07 ± 1.21 cm^2; p=0.001) and CON (24.75 ± 1.29 cm^2; p=0.004); VL mCSA was also greater for HIIT+EAA (27.26 ± 1.21 cm^2) than EAA (p<0.001) and CON (p=0.002). Analysis of changes scores showed significant increase in VL mCSA from weeks 0-4 for HIIT (Δ: 1.16 ± 0.51 cm^2; [0.13,2.18]) and HIIT+EAA (Δ: 1.73 ± 0.51 cm^2; [0.70,2.75]), a significant increase from weeks 4-8 for HIIT (Δ: 1.59 ± 0.45 cm^2; [0.70,2.49]), and from weeks 0-8 for HIIT (Δ: 2.73 ± 0.53 cm^2; [1.66,3.80]) and HIIT+EAA (Δ: 2.51 ± 0.55 cm^2; [1.42,3.61]) (Figure 8). These changes were significantly greater than changes observed with EAA and CON (p>0.05).

Muscle Quality

There was no significant interaction effect (p=0.626) for EI of the VL adjusted for subcutaneous fat thickness. There was a main effect for group (p=0.002), but not time (p=0.539). Muscle quality was significantly better for HIIT (adjusted mean±SE: 130.21 ± 1.83 a.u.; p=0.006) and HIIT+EAA (130.03 ± 1.86 a.u.; p=0.005) compared to EAA (139.06 ± 1.86 a.u.), as indicated by a lower EI (table 9). Analysis of change scores showed improvements in muscle quality for HIIT and HIIT+EAA from weeks 0-4 (Δ: HIIT: -7.47 ± 2.51 a.u.; [-12.49,-2.45]; HIIT+EAA: -5.51 ± 2.51 a.u.; [-10.52,-0.50]) and 0-8 (Δ: HIIT: -5.46 ± 2.68 a.u.; [-10.84,-0.09]; HIIT+EAA: -7.97 ± 2.76 a.u.; [-13.49,-2.45]); there were no significant changes during weeks 4-8. Changes for HIIT+EAA, but not HIIT, were significantly greater than EAA (Δ0-8: 4.02 ± 2.84 a.u.; [-1.66,9.70]; p=0.022).

Muscle Volume

There was no significant interaction effect (p=0.421) for MV of the VL; there was a main effect for group (p<0.001), but not time (p=0.238). HIIT (adjusted mean±SE: 614.69 ± 32.96 cm^3; p=0.001)

and HIIT+EAA (614.36 ± 32.99 cm^3; p=0.002) had significantly greater MV than EAA (572.21 ± 32.99 cm^3), but not CON (579.92 ± 34.22 cm^3; p=0.101-0.112). Analysis of change scores showed significant increases in MV from weeks 0-4 for HIIT (Δ: 41.90 ± 10.23 cm^3; [21.44,62.36]) and HIIT+EAA (Δ: 33.73 ± 10.25 cm^3; [13.24,54.23]) and from weeks 4-8 for HIIT+EAA (Δ: 26.90 ± 8.53 cm^3; [9.82,43.99]), resulting in significant increases in MV from weeks 0-8 for HIIT (Δ:54.50 ± 11.69 cm^3; [31.07,77.92]) and HIIT+EAA (Δ: 62.39 ± 12.05 cm^3; [38.26,86.52]). Changes between HIIT and HIIT+EAA were not significantly different (p=1.000).

Muscle Architecture

There was no significant interaction or main effects for FL or PA (p>0.05). Based on mean change scores, there were no significant changes in FL from weeks 0-4 or 4-8, but there was a significant increase in FL from weeks 0-8 for HIIT+EAA (Δ:0.35 ± 0.16 cm; [0.04,0.67]), but not HIIT (Δ: 0.21 ± 0.15 cm; [-0.10,0.52]). There were no changes in PA.

Whole Body Protein Turnover

In the subsample of individuals who completed the measure of whole body protein turnover (n=22), HIIT and EAA groups were considered to be in protein balance at baseline, with the HIIT+EAA group being in a slight positive balance (Mean±SD [95%CI]: 0.24 ± 0.23 g/kgBM/day, [0.03,0.45]). After adjusting for baseline values, net balance significantly decreased from weeks 0-8 for HIIT+EAA (Δ: -0.36 ± 0.16 g/kgBM/day; [-0.70,-0.02]) and EAA (-0.46 ± 0.16 g/kgBM/day, [-0.81,-0.12]). However, both groups remained in protein balance, with no difference in net balance between groups at 8 weeks (p=0.157) (Figure 10A). Protein synthesis significantly decreased from weeks 0-8 for HIIT (Δ: -1.03±0.48 g/kg/BM/day; [-2.04,-0.02]), resulting in greater protein synthesis for HIIT+EAA and EAA at 8 weeks compared to HIIT (p<0.05) (Figure 10B). Protein breakdown did not significantly change from weeks 0-8 for any group, but HIIT+EAA and EAA tended to have greater breakdown at 8weeks compared to HIIT (significant group effect: p=0.032) (Figure 10C). Flux did not significantly change from weeks 0-8 for

any group, but HIIT+EAA and EAA tended to have greater flux at 8 weeks compared to HIIT (significant group effect: p=0.024)(Figure 10D). There were no significant differences in 24-hr dietary intake or training volume between groups (p>0.05)(Table 10).

Sex Differences

There was no group × time × sex interaction for any outcome (p>0.05). There was a group × sex interaction for thighLM (p=0.003); post hoc analysis showed significant differences between men and women only for CON (p=0.010). In men, thigh LM with HIIT (p<0.001), EAA (p=0.017), and HIIT+EAA (p<0.001) compared to control; there were no differences in thighLM between groups for women (p>0.05). There was also a group × sex interaction for PA (p=0.009); post-hoc analysis showed significant differences between men and women in HIIT (p=0.001) and CON (p=0.043). There were no differences in PA between groups in men (p>0.05); in women, PA was greater in HIIT+EAA than HIIT (adjusted mean difference ± SE; [95%CI]: 2.08 ± 0.64°; [-3.80,-0.35]).

Based on mean change scores in men, total body LM increased with HIIT+EAA from 4-8 wks (Δ: 1.01 ± 0.37 kg; [0.27,1.74]) and from 0-8 wks (Δ: 1.37 ± 0.43 kg; [0.52,2.23]). Thigh LM followed a similar trend, increasing with HIIT+EAA from 4-8wks (Δ: 0.22 ± 0.06 kg; [0.10,0.34]) and 0-8 (Δ: 0.32 ± 0.07 kg; [0.17,0.46]) (Figure 11A); thigh LM also increased with HIIT from weeks 4-8 (Δ: 0.15 ± 0.06 kg; [0.03,0.28]) and 0-8 (Δ: 0.27 ± 0.08 kg; [0.12,0.42]) (Figure 11A). Muscle CSA of the VL increased with HIIT and HIIT+EAA from weeks 0-4 (Δ: HIIT: 2.07 ± 0.77 cm^2; [0.53,3.60]; HIIT+EAA: 1.61 ± 0.73 cm^2; [0.16,3.06]), 4-8 (Δ: HIIT: 2.09 ± 0.64 cm^2; [0.80,3.38]; HIIT+EAA: 1.59 ± 0.65 cm^2; [0.30,2.88]), and 0-8 (Δ: HIIT: 4.18 ± 0.76 cm^2; [2.66,5.70]; HIIT+EAA: 3.59 ± 0.76 cm^2; [2.07,5.11]) (Figure 11B). There were no changes for RF mCSA; there was a slight increase in VM mCSA for CON from 0-8 wks (Δ: 3.12 ± 1.14 cm^2; [0.83,5.41]). Muscle quality of the VL improved with HIIT+EAA from weeks 0-4 (Δ: -9.12 ± 3.73 a.u.; [-16.60,-1.65]). MV significantly increased from weeks 0-4 for HIIT (Δ: 51.63 ± 16.22 cm^3; [19.16,84.10]) and HIIT+EAA (Δ: 50.31 ± 14.53 cm^3; [21.22,79.39]), from weeks 4-8 with HIIT+EAA (Δ: 33.08 ± 13.36 cm^3; [6.29,59.88]), resulting in significant increases from weeks 0-8 for

HIIT (Δ: 65.71 ± 18.72 cm^3; [28.17,103.25]) and HIIT+EAA (Δ: 89.11 ± 17.99 cm^3; [53.02,125.20]) (Figure 11C). FL significantly increased from weeks 0-4 (Δ: 0.56 ± 0.20 cm; [0.15,0.96]) and 0-8 (Δ: 0.56 ± 0.22 cm; [0.12,1.01]) with HIIT+EAA; PA also increased from weeks 4-8 in CON (Δ: 2.10 ± 0.95°; [0.18,4.01]).

Based on mean change scores in women, there were no significant changes in total body LM, but there was an increase in thigh LM with HIIT+EAA from weeks 0-4 (Δ: 0.15 ± 0.06 kg; [0.03,0.28]). Muscle CSA of the RF (Δ: 1.01 ± 0.41 cm^2; [0.20,1.82]) and VL (Δ: 1.88 ± 0.76 cm^2; [0.35,3.40]) increased from weeks 0-4 with HIIT and HIIT+EAA, respectively; there were no other significant changes in mCSA. Muscle quality improved with HIIT (Δ: -8.22 ± 3.54 a.u.; [-15.30,-1.14]) from weeks 0-4 and declined with EAA (Δ: 11.35 ± 4.83 a.u.; [1.66,21.03]) from weeks 0-8. MV increased from weeks 0-4 (Δ: 33.19 ± 15.02 cm^3; [3.11,63.28]), and 0-8 (Δ: 44.09 ± 17.76 cm^3; [8.47,79.72]) with HIIT (Figure 11F); there were no changes from weeks 4-8 or with any other group. There were no changes in FL; PA decreased from weeks 0-8 for HIIT.

DISCUSSION:

Previous studies have suggested that HIIT may promote increases in lean body mass and muscle size [13-15, 34, 80, 114]. To date, these reports have been inconsistent, exploratory in nature, and have not included a nutritional arm. Results of the current study show that eight weeks of HIIT effectively increased LM size and quality, as indicated by increases in thigh LM, mCSA, MV, and EI, respectively. These improvements appear to be enhanced by EAA supplementation, via an increase in protein turnover. There were no significant differences in response between men and women. Men tended to have greater increases in LM in response to HIIT and HIIT+EAA, compared to women, but HIIT+EAA tended to support significant increases in women, compared to HIIT alone.

Previous studies exploring the effect of HIIT on LM have predominately focused on changes in total body composition, results of which have been relatively inconclusive as to the significance and magnitude of change induced by HIIT [48]. Inconsistencies are likely related to exercise modality; HIIT

training in the research setting is predominately conducted on a cycle ergometer, which almost exclusively targets the legs. The current study uniquely examined changes in thigh LM, estimating change in the muscles specifically being targeted by HIIT. Despite minimal changes in total body LM, HIIT and HIIT+EAA resulted in significant increases in thigh LM (HIIT: +0.17 kg; HIIT+EAA: +0.21 kg), specifically increasing mCSA (HIIT: +2.73 cm^2; HIIT+EAA: +2.51 cm^2) and MV (HIIT: +54.50 cm^3; HIIT+EAA: +62.39 cm^3) of the VL. Of the few studies that have evaluated regional changes in LM, significant increases in leg LM have been previously reported in overweight women (+0.4 kg) and men (+0.4 kg) after 6- and 12-weeks, respectively [34, 114]. After three weeks of HIIT, Blue et al. (2018) reported a non-significant 0.18 kg increase in leg LM and a significant 3.17 cm^2 increase in mCSA of the VL in overweight and obese men and women. Despite smaller increases in the current study, results are considered clinically significant and surpass measurement error. Age related loss of LM has previously been estimated to be around 1.9 kg and 1.1 kg per decade for men and women, respectively, with a greater percentage of loss occurring in the legs [146]. The increases in thigh LM observed in the study would effectively offset annual age-related declines in LM, which could have significant long-term impact for maintaining health, functionality, and quality of life.

Although changes in muscle size were not significantly different between HIIT and HIIT+EAA in the current study, results do suggest that EAA may support greater increases in LM. On average, increases in total body LM, thigh LM, and MV were greater for HIIT+EAA. Benefits of EAA supplementation in combination with HIIT are supported by analysis of whole-body protein turnover. Although all three groups (HIIT, EAA, and HIIT+EAA) remained in protein balance over the course of the intervention, when adjusting for baseline values, protein synthesis decreased with HIIT, while MPS was maintained with EAA. This resulted in greater MPS for HIIT+EAA and EAA compared to HIIT at 8-weeks. Muscle protein breakdown was also greater for HIIT+EAA and EAA at 8 weeks resulting in greater protein flux compared to HIIT only. This likely indicates greater protein turnover that is known to occur with increased availability of amino acids [60, 66]. It is important to note that these results were achieved, despite suboptimal dietary protein intake (0.9-1.0 g/kgBM/d) for building muscle mass (1.4-2.0

g/kg/d)[9]. Greater protein turnover would likely also benefit muscle quality, as energy needs for protein turnover are derived predominately from fat oxidation [60]. Both HIIT and HIIT+EAA resulted in significant improvements in muscle quality, as indicated by a decrease in echo intensity. Although not significantly different, improvements in muscle quality were greater for HIIT+EAA compared to HIIT, but muscle quality was not improved with EAA alone. Although whole-body protein flux was similar between EAA and HIIT+EAA, exercise is likely the more potent stimulator of improved muscle quality.

Changes in muscle size are often accompanied by changes in muscle architecture which are associated with muscle strength and force production [155]. Minimal changes in muscle architecture were observed in the current study, but there was a potential increase in FL after eight-weeks with HIIT+EAA. Using resistance training as a model, it has also been hypothesized that the high-intensity, rapid contractions of HIIT may damage contractile elements, inducing an acute inflammatory response and stimulating satellite cell repair, which could result in increased fiber length [18, 59, 155]. Muscle hypertrophy following high-volume resistance training has also been shown to be largely attributed to sarcoplasmic hypertrophy, as opposed to architectural changes [156]. Sarcoplasmic expansion was shown to be associated with increased proteins involved with glycolysis and ATP generation, which would have beneficial effects for HIIT performance [156]. It is currently unclear how sarcoplasmic expansion may influence strength/power outcomes, but further research into the mode of hypertrophy and concurrent influences on strength, functionality, and metabolic outcomes, as a result of HIIT is warranted.

An exploratory aim of this study was to evaluate the potential modulatory effect of sex on adaptation to HIIT. No interaction effects of sex on response to HIIT were found in the current study, suggesting minimal differential effects of sex on response to HIIT. Analysis of change scores showed significant responses predominately occurred in men, however, in women, significant increases in thigh LM and VL mCSA were observed with HIIT+EAA after four weeks, while VL MV increased from weeks 0-4 and 0-8 with HIIT. There is considerable debate as to whether males and females respond differently to HIIT [144]. Differences in response to moderate continuous exercise between men and women, typically favoring more positive responses in men, is predominately attributed to differences in

sex hormones [142, 157]. However, recent evidence in mice suggests that HIIT may overcome these differences, promoting positive changes in both men and women [78]. Although few studies in humans have directly evaluated sex differences in response to HIIT, a majority of studies also report no effect of sex on responses to HIIT [35, 81-88]. Specific to the current study, following 12-weeks of sprint-interval training (SIT), Heydari et al. (2012) reported significant increases in total body fat-free mass (1.2 kg) and increased LM in the legs and trunk in overweight men [54]. After 6-weeks of SIT, Gillen et al. (2013) reported a non-significant 0.6 kg average increase in total body LM in overweight and obese women. Although non-significant, if extrapolated out to 12-weeks, this gain in LM would be equivalent to the increase reported by Heydari et al. in men. Gillen et al. also reported a significant increases in leg LM (+0.4 kg) [34]. Scalzo et al. (2014) reported greater MPS and mitochondrial biogenesis in men compared to women following sprint-intervals, but no differences in oxygen consumption, time-trial performance, or power output were reported [35]. Greater MPS in men in the present study could support the significant increases in LM that were observed in men. However, in the present study HIIT also appeared to have beneficial effects in women, especially when EAA were provided.

In conclusion, significant increases in thigh LM and improved muscle quality can be achieved with eight weeks of HIIT training in overweight and obese adults. Twice daily EAA supplementation appears to support greater increases in LM, by increasing whole-body protein turnover. Results suggest that increases in thigh LM, mCSA, MV, and improved muscle quality can occur in as early as four weeks, adding to the growing body of evidence supporting unique benefits of HIIT as a time efficient and effective approach for improving health outcomes [12]. Benefits appear to extend to both men and women, with EAA potentially being especially important for supporting muscular changes in women. When combined with the significant improvements in cardiorespiratory fitness that are characteristic of HIIT, these results have significant implications as a potential approach for maintaining or improving LM in aging populations or those at risk for significant muscle wasting.

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CHAPTER VI

CONCLUSIONS

In conclusion, eight weeks of HIIT, with and without EAA, did not improve total body composition, but increased thigh LM size and muscle quality, while also promoting positive improvements in metabolic rate, fat oxidation, and cardiorespiratory fitness in overweight and obese men and women. Twice daily EAA consumption in combination with HIIT, supported greater increases in LM, and improved muscle quality, by increasing whole-body protein turnover. EAA also stimulated positive metabolic adaptations, increasing metabolic rate and fat oxidation, independent of exercise. Benefits appear to extend to both men and women, with EAA potentially being especially important for supporting muscular changes in women and promoting more rapid changes in men.

The current study provides evidence of beneficial effects of an exercise and nutrition intervention that is requires minimal training time and lifestyle changes. Results add to the growing body of evidence supporting unique benefits of HIIT as a time efficient approach for improving health outcomes. The improvements in cardiorespiratory fitness achieved in the first four weeks alone with HIIT+EAA (+3.7 ml/kg/min) have been shown to be associated with 11.6%, 16.1%, and 14.0% reduction in all-cause, cardiovascular disease, and cancer mortality [158], with even greater increases with HIIT (+5.1 ml/kg/min) and HIIT+EAA (+4.1 ml/kg/min) after eight weeks. Increases in thigh LM after eight weeks of HIIT (+0.17 kg) and HIIT+EAA (+0.22) was enough to offset annual age-related loss of LM [146]. Greater benefits, especially to total body composition, may be achieved with greater frequency of HIIT (3d/wk) and more involved dietary changes, namely caloric restriction and increased protein intake. However, when taken in context, improvements of the current study were achieved, with good compliance, in a population that could be considered relatively healthy, raising questions for the potential for benefits in more clinical populations.

TABLES

Table 1: Baseline Participant Characteristics (Mean ± SD)

TOTAL GROUP	**HIIT (N=19)**	**EAA (N=20)**	**HIIT+EAA (N=19)**	**CON (N=8)**
Age (yrs)	36.74 ± 5.61	37.20 ± 5.52	36.21 ± 6.65	36.88 ± 7.45
Height (cm)	173.76 ± 10.12	169.26 ± 8.91	170.64 ± 10.52	173.28 ± 9.51
Weight (kg)	96.57 ± 17.23	95.91 ± 13.19	91.78 ± 13.54	92.20 ± 15.52
BMI (kg/m2)	31.73 ± 4.72	33.52 ± 4.42	31.41 ± 3.36	30.55 ± 3.91
MALES	**HIIT (N=9)**	**EAA (N=10)**	**HIIT+EAA (N=10)**	**CON (N=4)**
Age (yrs)	36.67 ± 5.96	35.60 ± 4.95	37.30 ± 7.65	39.00 ± 10.80
Height (cm)	181.57 ± 6.09	175.51 ± 6.53	178.01 ± 8.11	180.63 ± 3.77
Weight (kg)	107.24 ± 13.02	96.66 ± 16.33	98.94 ± 10.07	101.48 ± 13.45
BMI (kg/m2)	32.69 ± 5.44	31.22 ± 4.29	31.14 ± 2.20	31.13 ± 5.00
FEMALES	**HIIT (N=10)**	**EAA (N=10)**	**HIIT+EAA (N=9)**	**CON (N=4)**
Age (yrs)	36.80 ± 5.59	38.80 ± 5.85	35.00 ± 5.52	34.75 ± 0.96
Height (cm)	166.74 ± 7.49	163.01 ± 6.18	162.46 ± 5.64	165.93 ± 7.25
Weight (kg)	86.97 ± 15.06	95.15 ± 9.95	83.82 ± 12.76	82.93 ± 12.33
BMI (kg/m2)	30.86 ± 4.07	35.82 ± 3.32	31.70 ± 4.45	29.98 ± 3.12

No differences between group ($p>0.05$)

Table 2: Body composition (Mean ± SD)

		HIIT	EAA	HIIT+EAA	CON
FM (kg)	Baseline	35.27 ± 9.52	36.58 ± 11.58	31.79 ± 9.17	32.43 ± 10.48
	4 weeks	35.40 ± 9.87	36.63 ± 11.69	32.00 ± 9.33	32.65 ± 10.33
	8 weeks	35.17 ± 10.01	34.82 ± 11.52	32.10 ± 9.14	32.59 ± 10.53
%BF	Baseline	36.23 ± 6.20	37.64 ± 9.49	34.52 ± 7.18	34.97 ± 8.37
	4 weeks	36.42 ± 6.71	37.64 ± 9.54	34.63 ± 7.18	35.21 ± 8.26
	8 weeks	36.21 ± 6.41	35.92 ± 9.19	34.78 ± 7.09	34.94 ± 8.81
FFM (kg)	Baseline	61.30 ± 10.86	59.33 ± 9.42	59.99 ± 10.50	59.77 ± 11.66
	4 weeks	61.03 ± 11.03	59.38 ± 9.47	59.95 ± 10.17	59.60 ± 11.30
	8 weeks	61.07 ± 10.80	60.59 ± 9.19	59.87 ± 10.84	60.16 ± 11.53
VAT (kg)	Baseline	1.33 ± 0.73	1.30 ± 0.61	1.16 ± 0.53	1.30 ± 0.54
	4 weeks	1.36 ± 0.76	1.30 ± 0.63	1.09 ± 0.51	1.33 ± 0.57
	8 weeks	1.38 ± 0.77	1.36 ± 0.63	1.16 ± 0.55	1.31 ± 0.56

No significant baseline differences ($p>0.05$); No significant interaction or main effects ($p>0.05$).

Table 3: Metabolic Characteristics (Mean ± SE)

		HIIT	EAA	HIIT+EAA	CON
RMR (kg/d)	Baseline	1793.37 ± 278.92	1718.45 ± 239.20	1709.32 ± 256.63	1757.50 ± 269.29
	4 weeks	1861.37 ± 277.99*	1774.30 ± 266.71*	1757.26 ± 256.64	1785.88 ± 286.92
	8 weeks	1872.68 ± 336.65[#]	1793.88 ± 266.93	1768.17 ± 274.91	1836.75 ± 266.21
RER (a.u.)	Baseline	0.77 ± 0.04	0.77 ± 0.04	0.80 ± 0.06	0.78 ± 0.04
	4 weeks	0.78 ± 0.06	0.75 ± 0.05	0.78 ± 0.04	0.77 ± 0.06
	8 weeks	0.74 ± 0.05[§#]	0.75 ± 0.04[#]	0.76 ± 0.06	0.77 ± 0.06

*No significant interaction or main effect of group ($p>0.05$); significant main effect of time for RER ($p=0.021$); significant change from *0-4wks, [§]4-8wks, and [#]0-8wks based on adjusted mean change ± 95% CI ($p<0.05$).*

Table 4: Cardiorespiratory fitness outcomes (Mean ± SD)

		HIIT	EAA	HIIT+EAA	CON
VO_{2rel} (ml/kg/min)	Baseline	28.02 ± 6.25	27.03 ± 7.52	30.41 ± 6.35	30.36 ± 8.59
	4 weeks	29.08 ± 6.03	28.45 ± 8.20	33.81 ± 7.66*	30.65 ± 7.17
	8 weeks	33.01 ± 7.30$^{\S\#}$	30.33 ± 8.93$^{\#}$	34.78 ± 7.69$^{\#}$	30.50 ± 9.98
VT (L/min)	Baseline	1.35 ± 0.40	1.28 ± 0.33	1.50 ± 0.35	1.40 ± 0.38
	4 weeks	1.39 ± 0.56	1.39 ± 0.42	1.47 ± 0.48	1.34 ± 0.36
	8 weeks	1.59 ± 0.49$^{\S\#}$	1.40 ± 0.33	1.74 ± 0.49$^{\S\#}$	1.46 ± 0.42

*No significant interaction ($p>0.05$); Significant main effect of group for VO_{2rel} ($p=0.002$) showing HIIT ($p=0.016$) and HIIT+EAA ($p=0.005$) greater than CON; significant change from *0-4wks, §4-8wks, and $^{\#}$0-8wks based on adjusted mean change ± 95% CI ($p<0.05$).*

Table 5: Cardiometabolic markers (Mean ± SD)

		HIIT	EAA	HIIT+EAA	CON
TC	Baseline	191.89 ± 32.59	186.47 ± 25.16	188.53 ± 40.66	193.88 ± 43.84
	8 weeks	187.05 ± 31.02	184.41 ± 29.56	185.00 ± 42.16	188.00 ± 42.69
HDL	Baseline	48.00 ± 16.19	48.05 ± 9.54	52.26 ± 8.82	44.00 ± 12.00
	8 weeks	47.42 ± 16.90	46.76 ± 10.99	51.78 ± 10.47	42.25 ± 9.07
nHDL	Baseline	143.89 ± 33.66	138.58 ± 28.96	136.21 ± 42.81	150.00 ± 41.80
	8 weeks	139.74 ± 31.01	137.65 ± 28.77	132.56 ± 43.63	145.63 ± 43.56
GLU	Baseline	91.95 ± 8.28	89.37 ± 8.80	90.58 ± 12.53	91.38 ± 6.72
	8 weeks	91.68 ± 11.87	93.41 ± 7.93	95.22 ± 15.36	91.88 ± 7.90

No significant baseline differences ($p>0.05$); No significant interaction or main effects ($p>0.05$).

Table 6: Dietary Intake (Mean ± SD)

		HIIT	EAA	HIIT+EAA	CON
Calories (kcal/d)	Baseline	2224.12 ± 467.71	1869.16 ± 748.23	1875.87 ± 599.83	2080.63 ± 493.73
	4 weeks	2044.24 ± 491.34	2120.74 ± 688.91	1970.52 ± 582.52	2118.54 ± 404.13
	8 weeks	2172.09 ± 776.38	2001.53 ± 699.06	2002.29 ± 626.22	2221.73 ± 487.69
CHO (g/d)	Baseline	245.48 ± 69.90	216.15 ± 118.48	222.91 ± 79.39	214.20 ± 38.62
	4 weeks	229.24 ± 73.88	239.24 ± 90.73	215.69 ± 68.09	218.29 ± 64.69
	8 weeks	228.12 ± 82.92	209.51 ± 98.23	232.16 ± 61.43	221.09 ± 60.28
FAT (g/d)	Baseline	92.85 ± 24.34	83.23 ± 37.94	70.89 ± 28.09	85.06 ± 32.16
	4 weeks	80.49 ± 20.55	85.95 ± 31.61	81.41 ± 33.41	80.71 ± 25.25
	8 weeks	95.84 ± 39.12	86.18 ± 31.38	79.10 ± 34.21	91.61 ± 26.64
PRO (g/d)	Baseline	90.64 ± 23.56	76.78 ± 26.08	77.45 ± 20.90	89.03 ± 36.60
	4 weeks	89.68 ± 22.33	95.52 ± 29.82	85.56 ± 26.42	90.29 ± 34.39
	8 weeks	92.91 ± 39.07	92.88 ± 30.83	82.09 ± 28.92	97.68 ± 47.27
Relative PRO (g/kg/d)	Baseline	0.96 ± 0.29	0.80 ± 0.26	0.86 ± 0.27	0.97 ± 0.35
	4 weeks	0.95 ± 0.24	1.01 ± 0.35	0.93 ± 0.25	0.97 ± 0.37
	8 weeks	0.98 ± 0.38	1.00 ± 0.40	0.90 ± 0.31	1.05 ± 0.46

No significant baseline differences ($p>0.05$); No significant interaction or main effects ($p>0.05$).

Table 7: Total body and regional lean mass (Mean ± SD)

		HIIT	EAA	HIIT+EAA	CON
LM (kg)	Baseline	55.72 ± 10.93	53.74 ± 9.31	53.99 ± 10.36	54.31 ± 12.36
	4 weeks	55.56 ± 10.75	54.04 ± 9.23	54.31 ± 10.25	54.25 ± 11.81
	8 weeks	55.57 ± 10.81	55.05 ± 9.36	54.38 ± 11.02[#]	54.45 ± 11.62
ThighLM (kg)	Baseline	7.25 ± 1.65	7.08 ± 1.37	7.09 ± 1.50	7.25 ± 2.03
	4 weeks	7.34 ± 1.72*	7.15 ± 1.39*	7.22 ± 1.53*	7.22 ± 1.93
	8 weeks	7.42 ± 1.73[§#]	7.26 ± 1.43	7.27 ± 1.61[§#]	7.24 ± 1.84

*No significant interaction (p>0.05); Significant main effect of group for thighLM (p=0.003) showing HIIT (p=0.035) and HIIT+EAA (p=0.003) greater than CON; p-values based on adjusted means covaried for baseline values; significant change from *0-4wks, [§]4-8wks, and [#]0-8wks based on adjusted mean change ± 95% CI (p<0.05).*

Table 8: Muscle cross sectional area of the superficial muscles of the quad (Mean ± SD)

		HIIT	EAA	HIIT+EAA	CON
RF (cm^2)	Baseline	11.08 ± 3.67	10.60 ± 2.24	10.59 ± 2.21	10.55 ± 2.03
	4 weeks	11.68 ± 4.50	10.49 ± 2.14	10.83 ± 2.82	10.86 ± 2.26
	8 weeks	11.49 ± 4.36	10.66 ± 3.05	10.92 ± 3.24	10.81 ± 2.23
VL (cm^2)	Baseline	24.69 ± 6.20	24.78 ± 6.24	25.12 ± 5.79	25.47 ± 7.31
	4 weeks	25.86 ± 7.02*	24.37 ± 6.21	26.84 ± 5.49*	25.33 ± 7.68
	8 weeks	27.43 ± 7.30$^{\S\#}$	26.39 ± 5.17	27.77 ± 6.28$^{\#}$	24.80 ± 7.15
VM (cm^2)	Baseline	21.24 ± 5.88	21.20 ± 6.16	19.10 ± 4.57	18.56 ± 7.58
	4 weeks	21.90 ± 5.85	20.64 ± 5.99	19.49 ± 5.31	19.12 ± 7.27
	8 weeks	21.70 ± 6.43	21.08 ± 6.81	19.73 ± 4.76	20.12 ± 8.23$^{\#}$

*No significant interaction ($p>0.05$); Significant main effect of group for VM ($p=0.044$); Significant main effect of group for VL ($p<0.001$) showing HIIT and HIIT+EAA greater than EAA ($p=0.001$; $p<0.001$) and CON ($p=0.004$; $p=0.002$); p-values based on adjusted means covaried for baseline values; significant change from *0-4wks, §4-8wks, and $^{\#}$0-8wks based on adjusted mean change ± 95% CI ($p<0.05$).*

Table 9: Muscle characteristics of the vastus lateralis (Mean ± SD)

		HIIT	EAA	HIIT+EAA	CON
EI (a.u.)	Baseline	132.96 ± 35.81	144.78 ± 45.83	137.03 ± 39.93	135.72 ± 43.03
	4 weeks	125.94 ± 31.60	145.38 ± 43.88	131.61 ± 41.29	129.62 ± 35.52
	8 weeks	127.54 ± 35.64	138.00 ± 42.47	130.32 ± 41.76	135.04 ± 42.06
FL (cm)	Baseline	7.07 ± 1.28	7.24 ± 1.11	7.19 ± 1.05	8.26 ± 0.51
	4 weeks	7.31 ± 1.21	7.42 ± 0.84	7.46 ± 1.10	8.10 ± 0.57
	8 weeks	7.35 ± 1.18	7.13 ± 0.75	7.57 ± 0.93$^{\#}$	7.95 ± 0.91
PA (°)	Baseline	18.08 ± 2.42	17.91 ± 3.10	18.98 ± 3.26	15.68 ± 3.31
	4 weeks	17.82 ± 3.09	17.04 ± 3.15	19.27 ± 3.16	15.59 ± 2.62
	8 weeks	17.67 ± 2.99	17.67 ± 2.10	19.15 ± 3.27	16.64 ± 3.59
MV (cm^3)	Baseline	567.32 ± 163.92	558.95 ± 145.14	544.74 ± 158.76	603.63 ± 211.28
	4 weeks	609.26 ±179.32*	568.57 ± 138.17	578.33 ± 182.94*	611.50 ± 190.34
	8 weeks	621.49 ± 189.77$^{\#}$	583.20 ± 145.60	609.09 ± 211.16$^{\S\#}$	625.74 ± 198.64

*No significant interaction ($p>0.05$); Significant main effect of group for EI ($p=0.002$) showing HIIT ($p=0.006$) and HIIT+EAA ($p=0.005$) better than EAA; Significant main effect of group for MV ($p<0.001$) showing HIIT ($p=0.001$) and HIIT+EAA ($p=0.002$) greater than EAA; p-values based on adjusted means covaried for baseline values; significant change from *0-4wks, §4-8wks, and $^{\#}$0-8wks based on adjusted mean change ± 95% CI ($p<0.05$).*

Table 10: 24-hr dietary intake for subsample of whole-body protein turnover (Mean ± SD)

		HIIT	EAA	HIIT+EAA
Calories (kcal/d)	Baseline	2139.83 ± 387.05	2166.02 ± 710.10	2060.20 ± 541.78
	4 weeks	2215.88 ± 518.96	2316.91 ± 741.44	2033.85 ± 787.58
	8 weeks	2308.24 ± 1026.84	2049.37 ± 852.85	2260.40 ± 783.38
CHO (g/d)	Baseline	242.85 ± 49.88	261.51 ± 152.24	241.79 ± 71.01
	4 weeks	264.62 ± 81.11	256.14 ± 114.86	211.54 ± 65.68
	8 weeks	234.52 ± 100.95	236.60 ± 137.44	244.07 ± 58.44
FAT (g/d)	Baseline	83.91 ± 19.52	96.80 ± 25.92	79.75 ± 30.00
	4 weeks	82.02 ± 23.36	96.55 ± 31.80	80.62 ± 52.48
	8 weeks	96.57 ± 49.85	79.00 ± 26.55	92.69 ± 47.85
PRO (g/d)	Baseline	86.87 ± 17.75	89.17 ± 22.75	74.41 ± 16.56
	4 weeks	91.04 ± 27.99	107.30 ± 31.23	89.44 ± 31.41
	8 weeks	104.81 ± 54.31	93.04 ± 28.63	92.88 ± 36.17
Relative PRO (g/kg/d)	Baseline	0.98 ± 0.20	0.90 ± 0.21	0.76 ± 0.19
	4 weeks	1.02 ± 0.30	1.09 ± 0.34	0.89 ± 0.24
	8 weeks	1.13 ± 0.47	0.94 ± 0.27	0.93 ± 0.36

No significant interaction or main effects ($p>0.05$).

FIGURES

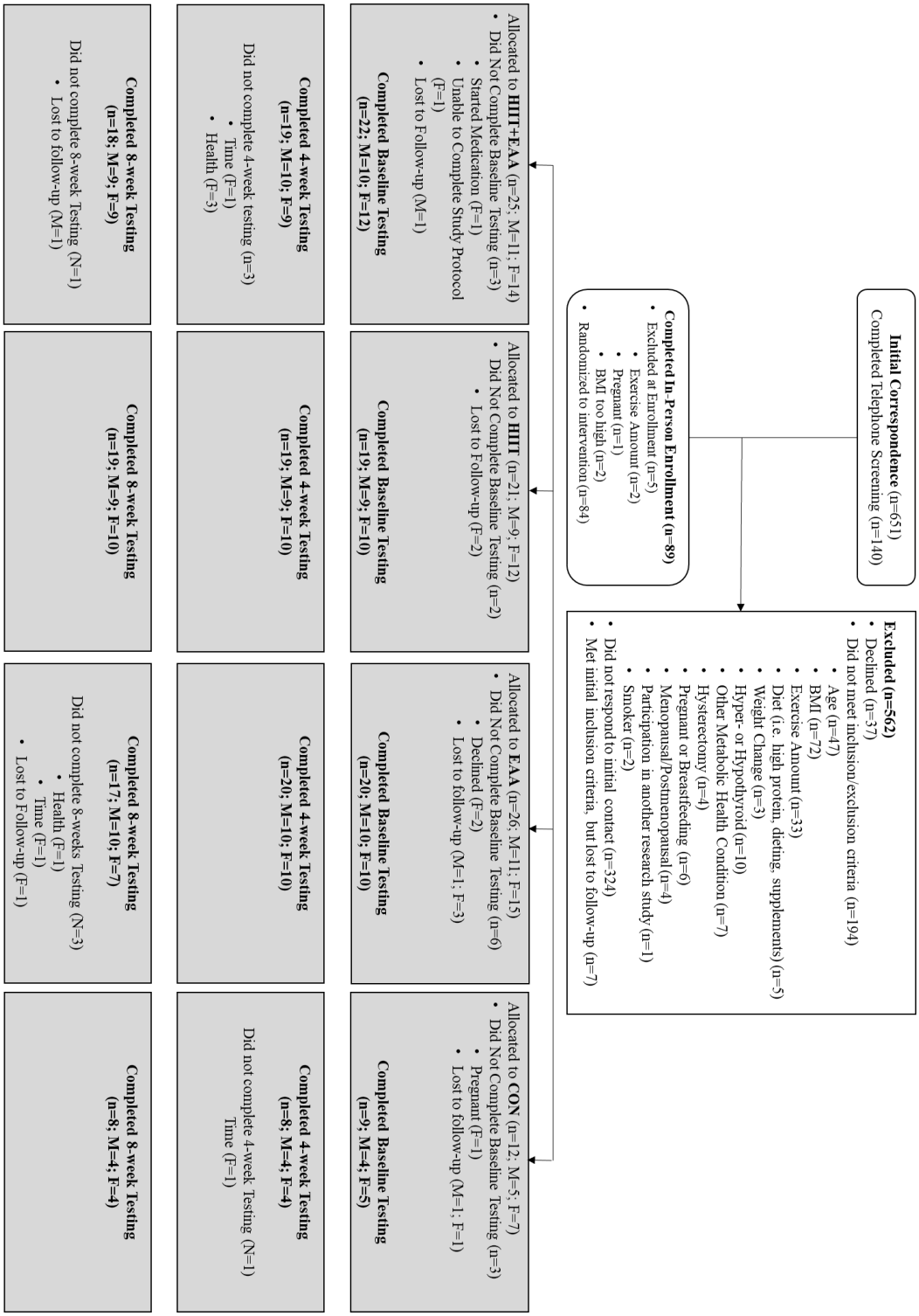

Figure 1: CONSORT guidelines

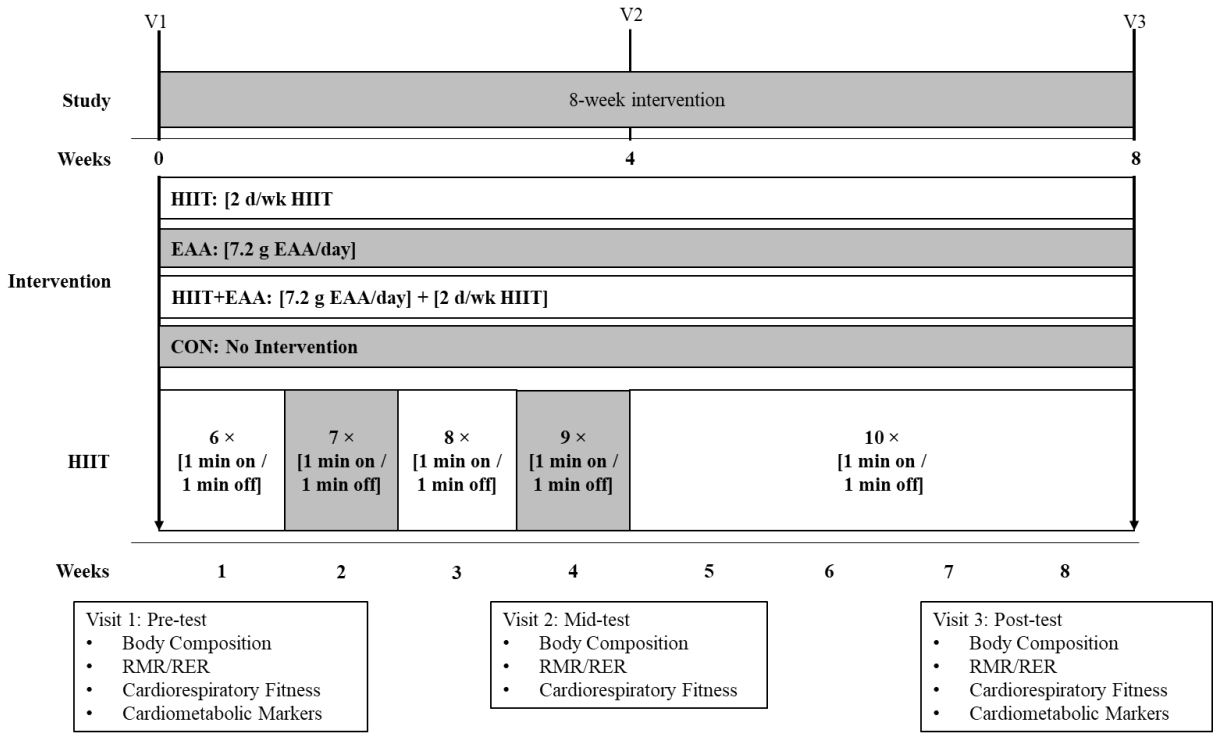

Figure 2: Experimental Design

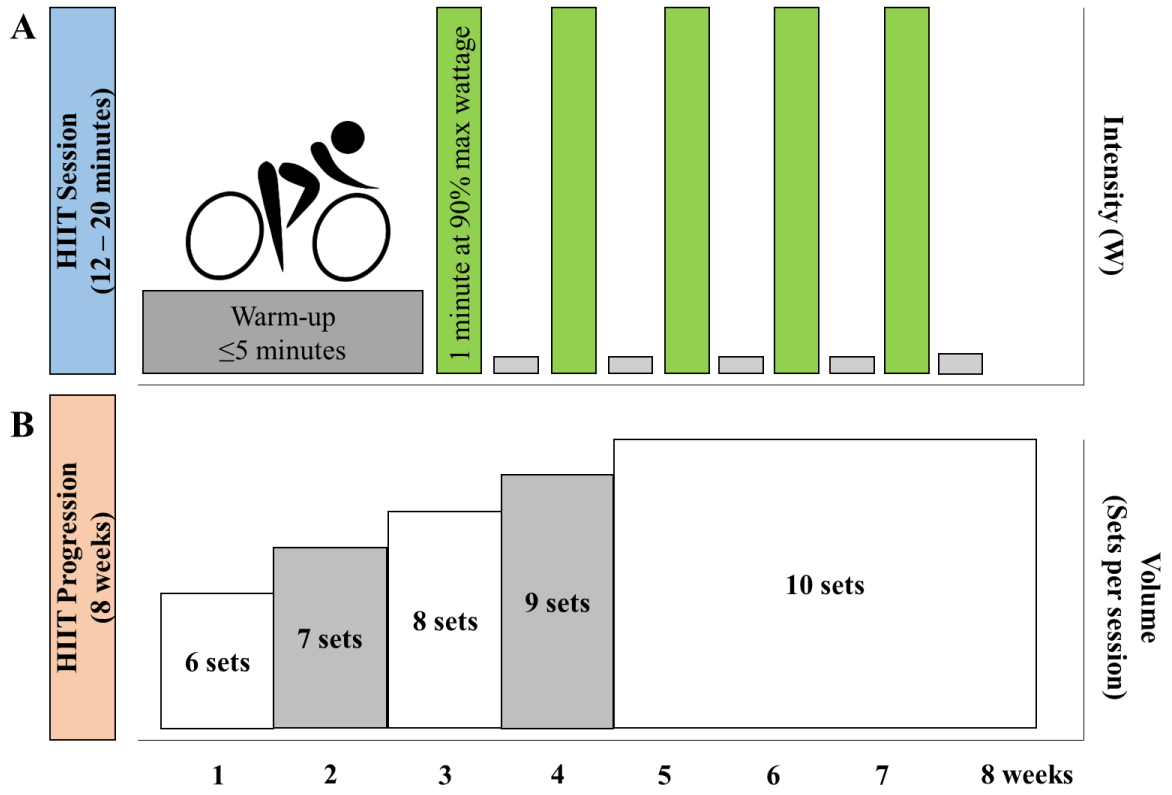

Figure 3: Timeline of HIIT session (A). Each session started with a self-selected warm-up, followed by alternating sets of one minute of hard pedaling (90% of max watts) and one-minute rest. During the final rep, individuals were asked to pedal as long as possible. If ride duration was ≥75 seconds total, resistance was increased by 7% at the next session; if the ride duration was <75 seconds, resistance was maintained for the next session. (B) Progression of HIIT over the course of the 8-week intervention. The intervention started with six sets of intervals. One set was added each week until reaching 10 sets at week five; 10 sets was maintained for the remainder of the 8-weeks.

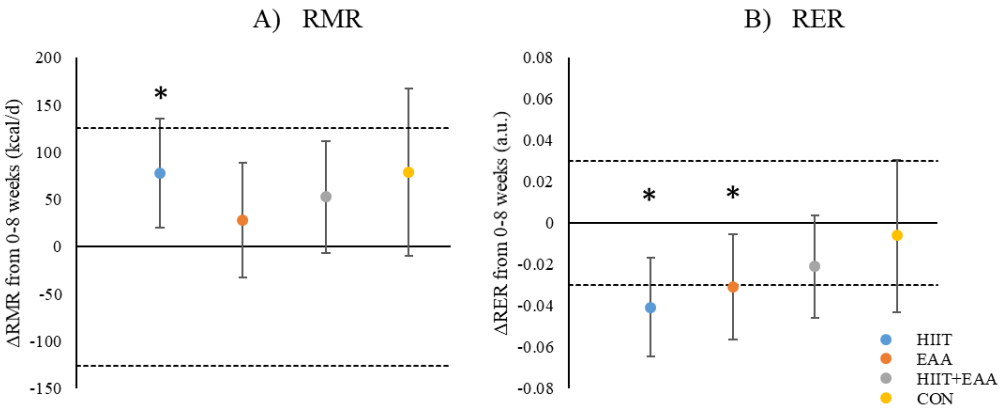

Figure 4: Change in a) RMR and b) RER from baseline to 8 weeks with 95% confidence intervals. Mean change scores are adjusted for baseline values. Dotted lines represent ±standard error of the measure of indirect calorimetry; *significant change based on 95% CI.

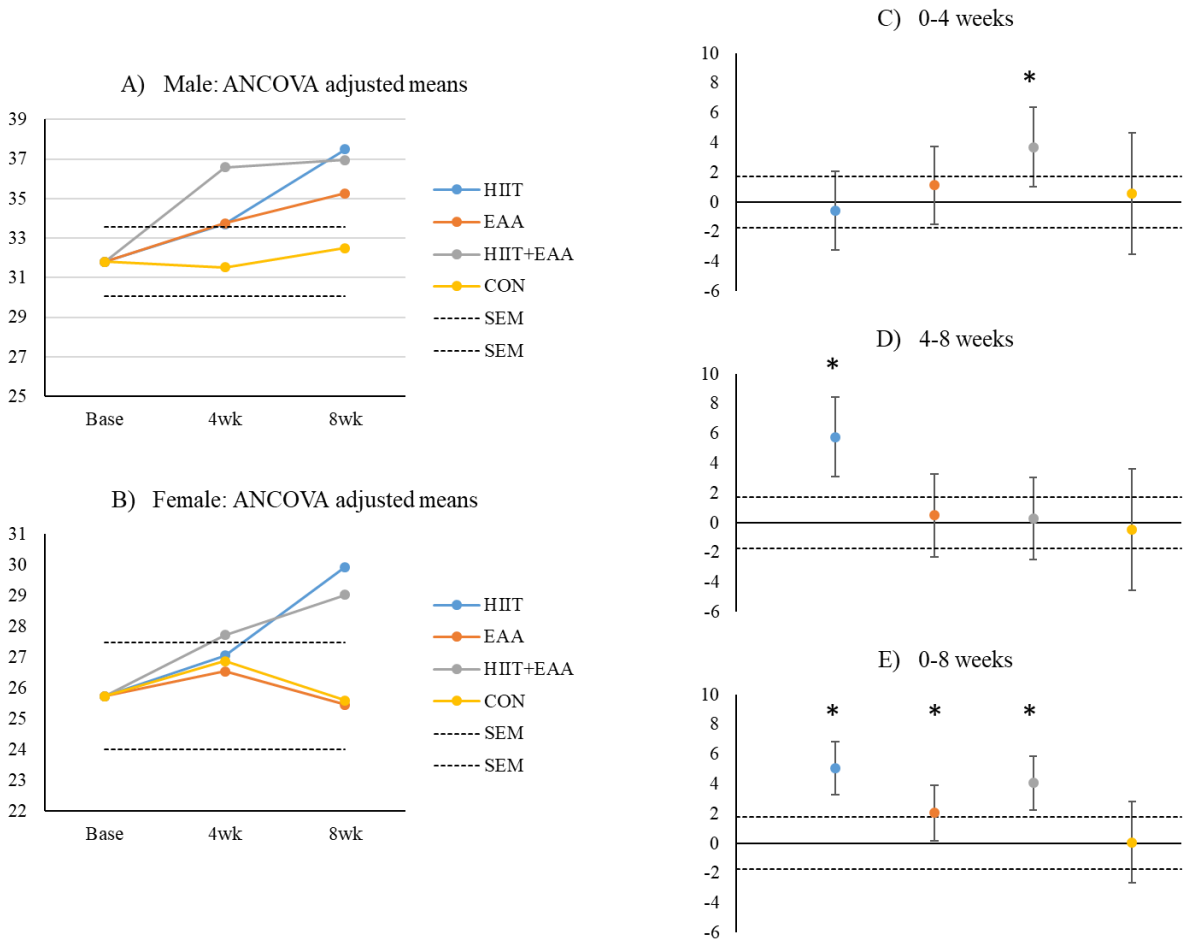

Figure 5: Adjusted mean relative VO2 (ml/kg/min) by group for a) men and b) women at base, 4week, and 8week. Adjusted mean change in relative VO2 with 95% confidence intervals by group (combined men and women) from c) 0-4 weeks, d) 4-8 weeks, e) 0-8 weeks. *significant change based on 95% CI.

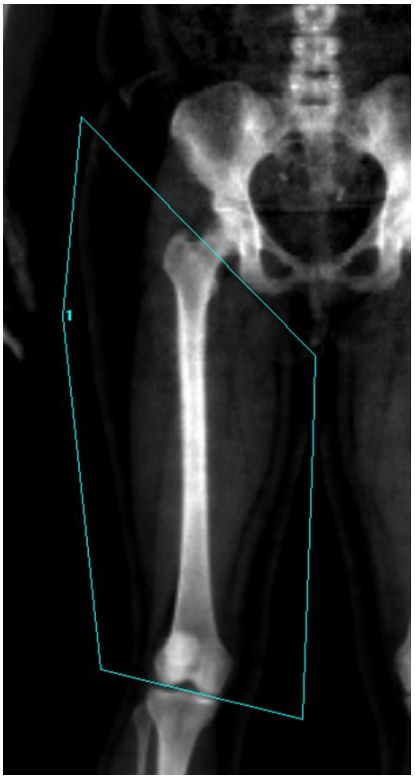

Figure 6: Thigh lean mass region-of-interest.

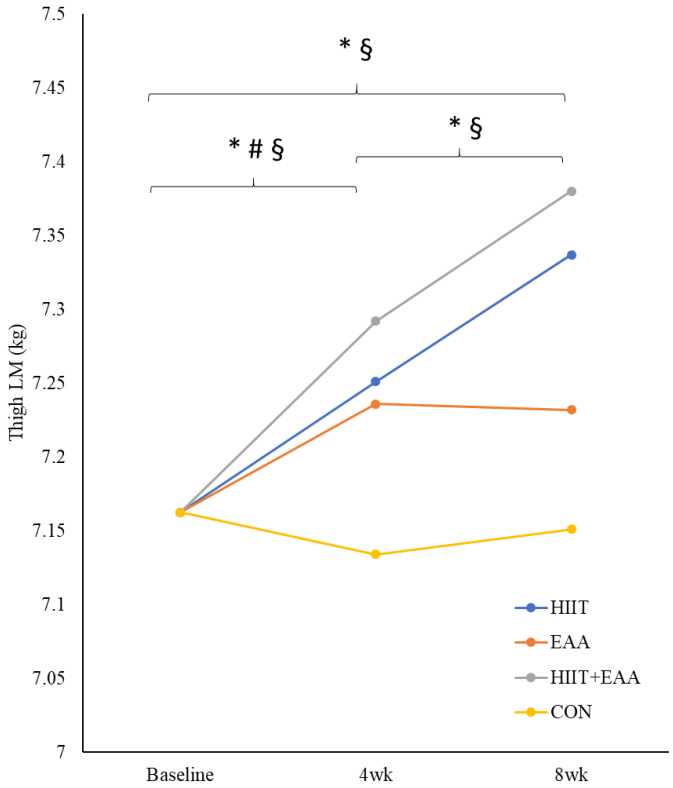

Figure 7: mean values presented are adjusted for baseline thigh LM (7.16 kg).
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#significant change for EAA based on adjusted mean change and 95% CI
§significant change for HIIT+EAA based on adjusted mean change and 95% CI

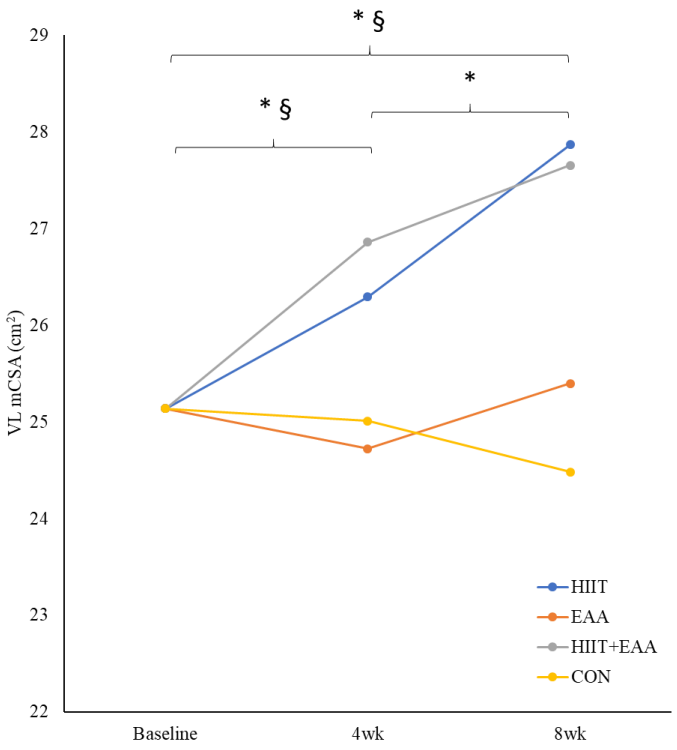

Figure 8: mean values presented are adjusted for baseline VL mCSA (25.14 cm^2).
*significant change for HIIT based in adjusted mean change and 95% CI
§significant change for HIIT+EAA based on adjusted mean change and 95% CI

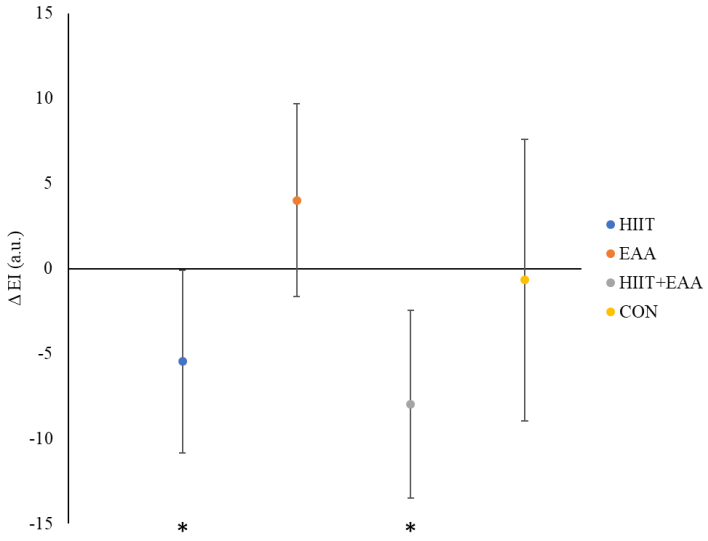

Figure 9: mean change and 95% CI values presented are adjusted for baseline VL EI (135.15 a.u.). *significant change from 0-8 weeks

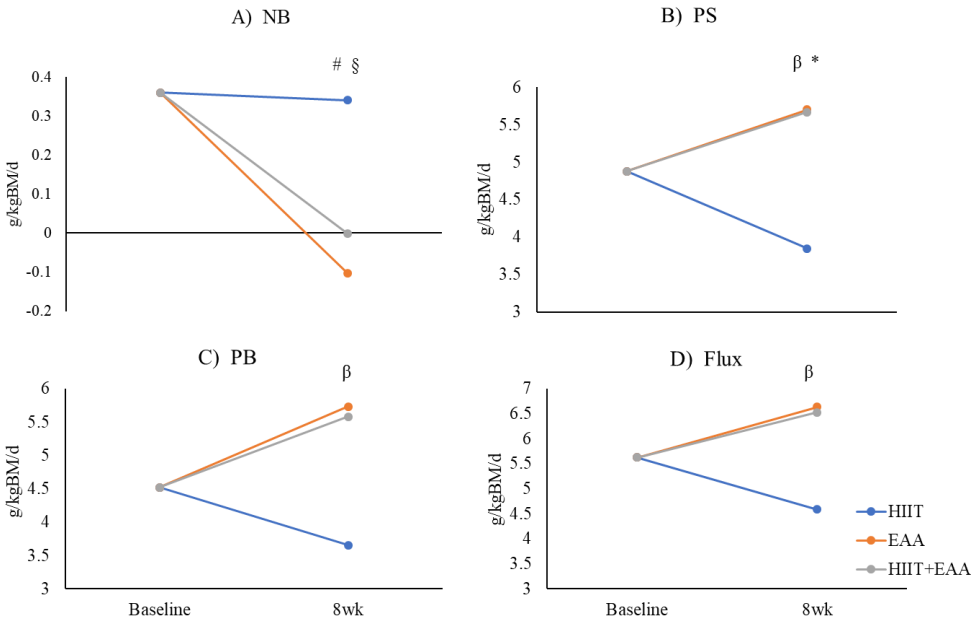

Figure 10: whole body protein turnover measures of net balance (A), protein synthesis (B), protein breakdown (C), and flux (D).
*significant change for HIIT based in adjusted mean change and 95% CI
#significant change for EAA based on adjusted mean change and 95% CI
§significant change for HIIT+EAA based on adjusted mean change and 95% CI
β significant main effect for group 8 weeks ($p<0.05$)

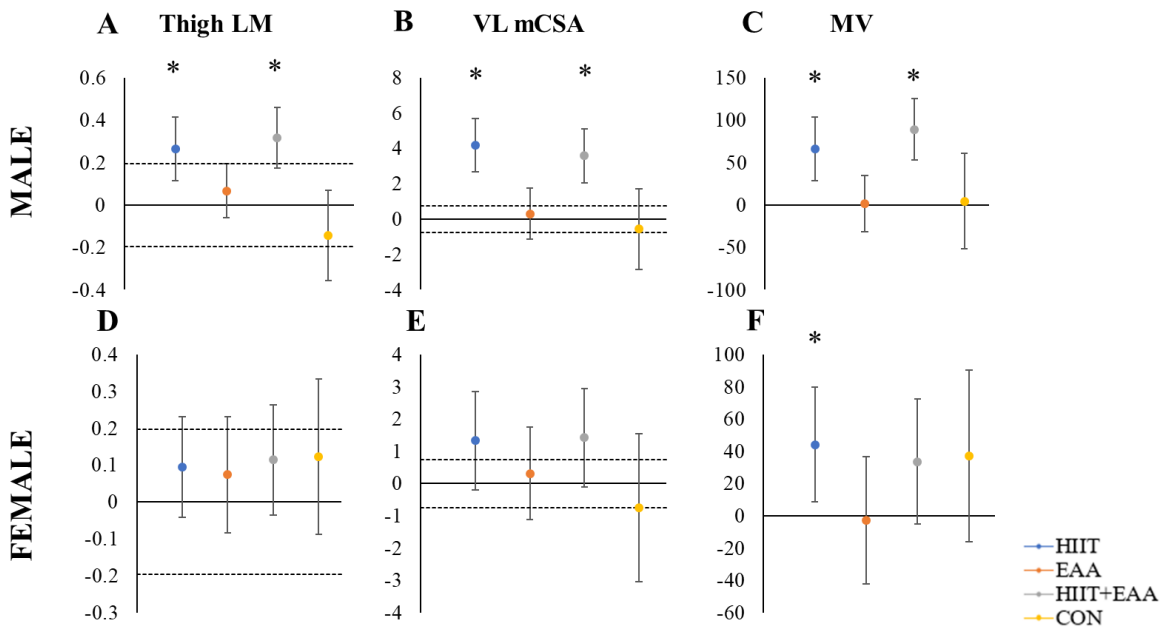

Figure 11: mean changes adjusted for baseline values with 95% CI values thigh LM, VL mCSA, and VM for men (A, B, C) and women (D, E, F); *significant change from 0-8 weeks; dotted lines represent standard error of the measure.

APPENDIX 1

Aim 2 of the originally proposed study included evaluation of metabolomic markers of fat oxidation and mitochondrial adaptation. These samples were collected, but due to limitations in funding, have not been analyzed. Mechanisms for potential funding have been identified and include: 1) the Translational Research and Matched Pilot Grant Program with the North Carolina Translational and Clinical Sciences Institute (NC TraCS); 2) a Pilot and Feasibility Project Grant through the Metabolomics Consortium Coordinating Center (M3C) together with the Southeast Center for Integrated Metabolomics (SECIM). KRH and ASR, have been in conversation KMH and with the North Carolina Nutrition Research Institute Metabolomics Core for analysis of the samples. Co-authors will be kept informed of progress of these applications and future analysis.

REFERENCES

1. Donnelly, J.E., et al., *American College of Sports Medicine Position Stand. Appropriate physical activity intervention strategies for weight loss and prevention of weight regain for adults.* Med Sci Sports Exerc, 2009. **41**(2): p. 459-71.

2. Piercy, K.L., et al., *The Physical Activity Guidelines for Americans.* Jama, 2018.

3. Arciero, P.J., et al., *Timed-daily ingestion of whey protein and exercise training reduces visceral adipose tissue mass and improves insulin resistance: the PRISE study.* J Appl Physiol (1985), 2014. **117**(1): p. 1-10.

4. Layman, D.K., et al., *A reduced ratio of dietary carbohydrate to protein improves body composition and blood lipid profiles during weight loss in adult women.* J Nutr, 2003. **133**(2): p. 411-7.

5. Layman, D.K., et al., *Dietary protein and exercise have additive effects on body composition during weight loss in adult women.* J Nutr, 2005. **135**(8): p. 1903-10.

6. Longland, T.M., et al., *Higher compared with lower dietary protein during an energy deficit combined with intense exercise promotes greater lean mass gain and fat mass loss: a randomized trial.* Am J Clin Nutr, 2016. **103**(3): p. 738-46.

7. Thomas, D.T., K.A. Erdman, and L.M. Burke, *American College of Sports Medicine Joint Position Statement. Nutrition and Athletic Performance.* Medicine and science in sports and exercise, 2016. **48**(3): p. 543-68.

8. Layman, D.K., et al., *Defining meal requirements for protein to optimize metabolic roles of amino acids.* Am J Clin Nutr, 2015.

9. Jager, R., et al., *International Society of Sports Nutrition Position Stand: protein and exercise.* Journal of the International Society of Sports Nutrition, 2017. **14**: p. 20.

10. Haskell, W.L., et al., *Physical activity and public health: updated recommendation for adults from the American College of Sports Medicine and the American Heart Association.* Circulation, 2007. **116**(9): p. 1081-93.

11. Gibala, M.J., J.B. Gillen, and M.E. Percival, *Physiological and health-related adaptations to low-volume interval training: influences of nutrition and sex.* Sports Med, 2014. **44 Suppl 2**: p. S127-37.

12. Gibala, M.J., et al., *Physiological adaptations to low-volume, high-intensity interval training in health and disease.* The Journal of physiology, 2012. **590**(5): p. 1077-84.

13. Smith-Ryan, A.E., et al., *Effects of high-intensity interval training on cardiometabolic risk factors in overweight/obese women.* J Sports Sci, 2016. **34**(21): p. 2038-46.

14. Blue, M.N.M., et al., *The effects of high intensity interval training on muscle size and quality in overweight and obese adults.* J Sci Med Sport, 2017.

15. Smith-Ryan, A.E., M.N. Melvin, and H.L. Wingfield, *High-intensity interval training: Modulating interval duration in overweight/obese men.* Phys Sportsmed, 2015. **43**(2): p. 107-13.

16. Wingfield, H.L., et al., *The acute effect of exercise modality and nutrition manipulations on post-exercise resting energy expenditure and respiratory exchange ratio in women: a randomized trial.* Sports Med Open, 2015. **1**(1): p. 11.

17. Boutcher, S.H., *High-intensity intermittent exercise and fat loss.* Journal of obesity, 2011. **2011**: p. 868305.

18. Bell, K.E., et al., *Day-to-Day Changes in Muscle Protein Synthesis in Recovery From Resistance, Aerobic, and High-Intensity Interval Exercise in Older Men.* The journals of gerontology. Series A, Biological sciences and medical sciences, 2015. **70**(8): p. 1024-9.

19. Hulmi, J.J., C.M. Lockwood, and J.R. Stout, *Effect of protein/essential amino acids and resistance training on skeletal muscle hypertrophy: A case for whey protein.* Nutrition & metabolism, 2010. **7**: p. 51.

20. Tarnopolsky, M.A. and B.C. Ruby, *Sex differences in carbohydrate metabolism.* Curr Opin Clin Nutr Metab Care, 2001. **4**(6): p. 521-6.

21. Shah, N.R. and E.R. Braverman, *Measuring adiposity in patients: the utility of body mass index (BMI), percent body fat, and leptin.* PLoS One, 2012. **7**(4): p. e33308.

22. Hirsch, K.R., et al., *Body Composition and Muscle Characteristics of Division I Track and Field Athletes.* J Strength Cond Res, 2016. **30**(5): p. 1231-8.

23. Melvin, M.N., et al., *Muscle characteristics and body composition of NCAA division I football players.* J Strength Cond Res, 2014. **28**(12): p. 3320-9.

24. Ryan, E.D., et al., *The influence of subcutaneous fat on the relationship between body composition and ultrasound-derived muscle quality.* Appl Physiol Nutr Metab, 2016. **41**(10): p. 1104-1107.

25. Morse, C.I., et al., *In vivo physiological cross-sectional area and specific force are reduced in the gastrocnemius of elderly men.* Journal of applied physiology, 2005. **99**(3): p. 1050-1055.

26. Gerstner, G.R., et al., *Neural and Muscular Contributions to the Age-Related Reductions in Rapid Strength.* Medicine and science in sports and exercise, 2017. **49**(7): p. 1331-1339.

27. Morse, C.I., H. Degens, and D.A. Jones, *The validity of estimating quadriceps volume from single MRI cross-sections in young men.* European journal of applied physiology, 2007. **100**(3): p. 267-274.

28. Shah, S.H., W.E. Kraus, and C.B. Newgard, *Metabolomic profiling for the identification of novel biomarkers and mechanisms related to common cardiovascular diseases: form and function.* Circulation, 2012. **126**(9): p. 1110-1120.

29. Jensen, M.D., et al., *2013 AHA/ACC/TOS guideline for the management of overweight and obesity in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and The Obesity Society.* Journal of the American college of cardiology, 2014. **63**(25 Part B): p. 2985-3023.

30. Seagle, H.M., et al., *Position of the American Dietetic Association: weight management.* Journal of the American Dietetic Association, 2009. **109**(2): p. 330-346.

31. Willis, L.H., et al., *Effects of aerobic and/or resistance training on body mass and fat mass in overweight or obese adults.* Journal of applied physiology, 2012. **113**(12): p. 1831-1837.

32. Bateman, L.A., et al., *Comparison of aerobic versus resistance exercise training effects on metabolic syndrome (from the Studies of a Targeted Risk Reduction Intervention Through Defined Exercise-STRRIDE-AT/RT).* The American journal of cardiology, 2011. **108**(6): p. 838-844.

33. Little, J.P., et al., *A practical model of low-volume high-intensity interval training induces mitochondrial biogenesis in human skeletal muscle: potential mechanisms.* The Journal of physiology, 2010. **588**(6): p. 1011-1022.

34. Gillen, J.B., et al., *Interval training in the fed or fasted state improves body composition and muscle oxidative capacity in overweight women.* Obesity, 2013. **21**(11): p. 2249-2255.

35. Scalzo, R.L., et al., *Greater muscle protein synthesis and mitochondrial biogenesis in males compared with females during sprint interval training.* FASEB J, 2014. **28**(6): p. 2705-14.

36. Laursen, P.B. and D.G. Jenkins, *The scientific basis for high-intensity interval training.* Sports medicine, 2002. **32**(1): p. 53-73.

37. Burgomaster, K.A., et al., *Six sessions of sprint interval training increases muscle oxidative potential and cycle endurance capacity in humans.* J Appl Physiol (1985), 2005. **98**(6): p. 1985-90.

38. Gibala, M.J., et al., *Short-term sprint interval versus traditional endurance training: similar initial adaptations in human skeletal muscle and exercise performance.* J Physiol, 2006. **575**(Pt 3): p. 901-11.

39. Jelleyman, C., et al., *The effects of high-intensity interval training on glucose regulation and insulin resistance: a meta-analysis.* Obes Rev, 2015. **16**(11): p. 942-61.

40. Weston, K.S., U. Wisløff, and J.S. Coombes, *High-intensity interval training in patients with lifestyle-induced cardiometabolic disease: a systematic review and meta-analysis.* Br J Sports Med, 2014. **48**(16): p. 1227-1234.

41. MacInnis, M.J. and M.J. Gibala, *Physiological adaptations to interval training and the role of exercise intensity.* The Journal of physiology, 2017. **595**(9): p. 2915-2930.

42. Burgomaster, K.A., et al., *Similar metabolic adaptations during exercise after low volume sprint interval and traditional endurance training in humans.* J Physiol, 2008. **586**(1): p. 151-60.

43. Huffman, K.M., et al., *Metabolite signatures of exercise training in human skeletal muscle relate to mitochondrial remodelling and cardiometabolic fitness.* Diabetologia, 2014. **57**(11): p. 2282-95.

44. Burgomaster, K.A., G.J. Heigenhauser, and M.J. Gibala, *Effect of short-term sprint interval training on human skeletal muscle carbohydrate metabolism during exercise and time-trial performance.* J Appl Physiol (1985), 2006. **100**(6): p. 2041-7.

45. Tomlin, D.L. and H.A. Wenger, *The relationship between aerobic fitness and recovery from high intensity intermittent exercise.* Sports Medicine, 2001. **31**(1): p. 1-11.

46. Skelly, L.E., et al., *High-intensity interval exercise induces 24-h energy expenditure similar to traditional endurance exercise despite reduced time commitment.* Applied Physiology, Nutrition, and Metabolism, 2014. **39**(7): p. 845-848.

47. Nalbandian, H.M., Z. Radak, and M. Takeda, *Effects of active recovery during interval training on plasma catecholamines and insulin.* The Journal of sports medicine and physical fitness, 2018. **58**(6): p. 917-922.

48. Wewege, M., et al., *The effects of high-intensity interval training vs. moderate-intensity continuous training on body composition in overweight and obese adults: a systematic review and meta-analysis.* Obesity Reviews, 2017. **18**(6): p. 635-646.

49. Trost, S.G., et al., *Correlates of adults' participation in physical activity: review and update.* Medicine & science in sports & exercise, 2002. **34**(12): p. 1996-2001.

50. Welch, N., et al., *Is the perception of time pressure a barrier to healthy eating and physical activity among women?* Public health nutrition, 2009. **12**(7): p. 888-895.

51. Keating, S., et al., *A systematic review and meta-analysis of interval training versus moderate-intensity continuous training on body adiposity.* Obesity Reviews, 2017. **18**(8): p. 943-964.

52. Sawyer, B.J., et al., *Effects of high-intensity interval training and moderate-intensity continuous training on endothelial function and cardiometabolic risk markers in obese adults.* Journal of Applied Physiology, 2016. **121**(1): p. 279-288.

53. Trapp, E.G., et al., *The effects of high-intensity intermittent exercise training on fat loss and fasting insulin levels of young women.* Int J Obes (Lond), 2008. **32**(4): p. 684-91.

54. Heydari, M., Y.N. Boutcher, and S.H. Boutcher, *The effects of high-intensity intermittent exercise training on cardiovascular response to mental and physical challenge.* International journal of psychophysiology : official journal of the International Organization of Psychophysiology, 2013. **87**(2): p. 141-6.

55. Di Donato, D.M., et al., *Influence of aerobic exercise intensity on myofibrillar and mitochondrial protein synthesis in young men during early and late postexercise recovery.* American journal of physiology. Endocrinology and metabolism, 2014. **306**(9): p. E1025-32.

56. Schoenfeld, B.J., *The mechanisms of muscle hypertrophy and their application to resistance training.* J Strength Cond Res, 2010. **24**(10): p. 2857-72.

57. Martinez-Valdes, E., et al., *Differential Motor Unit Changes after Endurance or High-Intensity Interval Training.* Med Sci Sports Exerc, 2017. **49**(6): p. 1126-1136.

58. Duchateau, J., J.G. Semmler, and R.M. Enoka, *Training adaptations in the behavior of human motor units.* Journal of Applied Physiology, 2006. **101**(6): p. 1766-1775.

59. Nederveen, J., et al., *The effect of exercise mode on the acute response of satellite cells in old men.* Acta Physiologica, 2015. **215**(4): p. 177-190.

60. Wolfe, R.R., *The underappreciated role of muscle in health and disease.* Am J Clin Nutr, 2006. **84**(3): p. 475-82.

61. Rasmussen, B.B. and R.R. Wolfe, *Regulation of fatty acid oxidation in skeletal muscle.* Annu Rev Nutr, 1999. **19**: p. 463-84.

62. Johns, D.J., et al., *Diet or exercise interventions vs combined behavioral weight management programs: a systematic review and meta-analysis of direct comparisons.* J Acad Nutr Diet, 2014. **114**(10): p. 1557-68.

63. Schwingshackl, L., S. Dias, and G. Hoffmann, *Impact of long-term lifestyle programmes on weight loss and cardiovascular risk factors in overweight/obese participants: a systematic review and network meta-analysis.* Syst Rev, 2014. **3**: p. 130.

64. Layman, D.K., et al., *Increased dietary protein modifies glucose and insulin homeostasis in adult women during weight loss.* J Nutr, 2003. **133**(2): p. 405-10.

65. Aragon, A.A., et al., *International society of sports nutrition position stand: diets and body composition.* J Int Soc Sports Nutr, 2017. **14**: p. 16.

66. Wolfe, R.R., *Effects of amino acid intake on anabolic processes.* Canadian Journal of Applied Physiology, 2001. **26**(S1): p. S220-S227.

67. Layman, D.K. and J.I. Baum, *Dietary protein impact on glycemic control during weight loss.* J Nutr, 2004. **134**(4): p. 968S-73S.

68. Rasmussen, B.B., et al., *An oral essential amino acid-carbohydrate supplement enhances muscle protein anabolism after resistance exercise*. Journal of applied physiology, 2000. **88**(2): p. 386-92.

69. Tipton, K.D., et al., *Timing of amino acid-carbohydrate ingestion alters anabolic response of muscle to resistance exercise.* American journal of physiology. Endocrinology and metabolism, 2001. **281**(2): p. E197-206.

70. Areta, J.L., et al., *Timing and distribution of protein ingestion during prolonged recovery from resistance exercise alters myofibrillar protein synthesis.* J Physiol, 2013. **591**(9): p. 2319-31.

71. Bartlett, J.D., J.A. Hawley, and J.P. Morton, *Carbohydrate availability and exercise training adaptation: too much of a good thing?* European journal of sport science, 2015. **15**(1): p. 3-12.

72. Marquet, L.A., et al., *Enhanced Endurance Performance by Periodization of Carbohydrate Intake: "Sleep Low" Strategy.* Medicine and science in sports and exercise, 2016. **48**(4): p. 663-72.

73. Taylor, C., et al., *Protein ingestion does not impair exercise-induced AMPK signalling when in a glycogen-depleted state: implications for train-low compete-high.* European journal of applied physiology, 2013. **113**(6): p. 1457-68.

74. Tappy, L., *Thermic effect of food and sympathetic nervous system activity in humans.* Reproduction Nutrition Development, 1996. **36**(4): p. 391-397.

75. Westerterp-Plantenga, M.S., et al., *Sex differences in energy homeostatis following a diet relatively high in protein exchanged with carbohydrate, assessed in a respiration chamber in humans.* Physiol Behav, 2009. **97**(3-4): p. 414-9.

76. Donnelly, J.E., et al., *Aerobic exercise alone results in clinically significant weight loss for men and women: midwest exercise trial 2.* Obesity, 2013. **21**(3): p. E219-E228.

77. Herrmann, S.D., et al., *Energy intake, nonexercise physical activity, and weight loss in responders and nonresponders: The Midwest Exercise Trial 2.* Obesity, 2015. **23**(8): p. 1539-1549.

78. McMullan, R.C., et al., *CC 002/Unc females are mouse models of exercise-induced paradoxical fat response.* Physiological Reports, 2018. **6**(12): p. e13716.

79. Weber, C.L. and D.A. Schneider, *Increases in maximal accumulated oxygen deficit after high-intensity interval training are not gender dependent.* Journal of applied physiology, 2002. **92**(5): p. 1795-801.

80. Macpherson, R.E., et al., *Run sprint interval training improves aerobic performance but not maximal cardiac output.* Medicine and science in sports and exercise, 2011. **43**(1): p. 115-22.

81. Bagley, L., et al., *Sex differences in the effects of 12 weeks sprint interval training on body fat mass and the rates of fatty acid oxidation and VO2max during exercise.* BMJ open sport & exercise medicine, 2016. **2**(1): p. e000056.

82. Metcalfe, R.S., et al., *Towards the minimal amount of exercise for improving metabolic health: beneficial effects of reduced-exertion high-intensity interval training.* European journal of applied physiology, 2012. **112**(7): p. 2767-75.

83. Gillen, J.B., et al., *Three minutes of all-out intermittent exercise per week increases skeletal muscle oxidative capacity and improves cardiometabolic health.* PLoS One, 2014. **9**(11): p. e111489.

84. Metcalfe, R.S., et al., *Changes in aerobic capacity and glycaemic control in response to reduced-exertion high-intensity interval training (REHIT) are not different between sedentary men and women.* Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme, 2016. **41**(11): p. 1117-1123.

85. Astorino, T.A., et al., *Adaptations to high-intensity training are independent of gender.* European journal of applied physiology, 2011. **111**(7): p. 1279-86.

86. Astorino, T.A. and M.M. Schubert, *Changes in fat oxidation in response to various regimes of high intensity interval training (HIIT).* European journal of applied physiology, 2018. **118**(1): p. 51-63.

87. Skelly, L.E., et al., *Effect of sex on the acute skeletal muscle response to sprint interval exercise.* Exp Physiol, 2017. **102**(3): p. 354-365.

88. Richards, J.C., et al., *Short-term sprint interval training increases insulin sensitivity in healthy adults but does not affect the thermogenic response to β-adrenergic stimulation.* The Journal of physiology, 2010. **588**(15): p. 2961-2972.

89. Tarnopolsky, M.A., *Sex differences in exercise metabolism and the role of 17-beta estradiol.* Medicine and science in sports and exercise, 2008. **40**(4): p. 648-54.

90. Torres, M.J., et al., *Impact of 17beta-estradiol on complex I kinetics and H2O2 production in liver and skeletal muscle mitochondria.* The Journal of biological chemistry, 2018. **293**(43): p. 16889-16898.

91. Klinge, C.M., *Estrogenic control of mitochondrial function and biogenesis.* Journal of cellular biochemistry, 2008. **105**(6): p. 1342-1351.

92. Kendrick, Z.V. and G.S. Ellis, *Effect of estradiol on tissue glycogen metabolism and lipid availability in exercised male rats.* Journal of Applied Physiology, 1991. **71**(5): p. 1694-1699.

93. Huffman, K.M., et al., *Impact of hormone replacement therapy on exercise training–induced improvements in insulin action in sedentary overweight adults.* Metabolism, 2008. **57**(7): p. 888-895.

94. Devries, M.C., et al., *17β-estradiol supplementation decreases glucose rate of appearance and disappearance with no effect on glycogen utilization during moderate intensity exercise in men.* The Journal of Clinical Endocrinology & Metabolism, 2005. **90**(11): p. 6218-6225.

95. Henderson, G.C. and B.L. Alderman, *Determinants of resting lipid oxidation in response to a prior bout of endurance exercise.* Journal of applied physiology, 2013. **116**(1): p. 95-103.

96. Burke, L.M. and J.A. Hawley, *Swifter, higher, stronger: What's on the menu?* Science, 2018. **362**(6416): p. 781-787.

97. Trexler, E.T., et al., *Dietary macronutrient distribution influences postexercise substrate utilization in women: a cross-sectional evaluation of metabolic flexibility.* The Journal of sports medicine and physical fitness, 2017. **57**(5): p. 580-588.

98. Smith-Ryan, A.E., et al., *Validity and reliability of a 4-compartment body composition model using dual energy x-ray absorptiometry-derived body volume.* Clin Nutr, 2017. **36**(3): p. 825-830.

99. Kaul, S., et al., *Dual-energy X-ray absorptiometry for quantification of visceral fat.* Obesity (Silver Spring), 2012. **20**(6): p. 1313-8.

100. Hogrel, J.Y., et al., *NMR imaging estimates of muscle volume and intramuscular fat infiltration in the thigh: variations with muscle, gender, and age.* Age, 2015. **37**(3): p. 9798.

101. Young, H.J., et al., *Measurement of intramuscular fat by muscle echo intensity.* Muscle Nerve, 2015. **52**(6): p. 963-71.

102. Ferrando, A.A., et al., *Prolonged bed rest decreases skeletal muscle and whole body protein synthesis.* Am J Physiol, 1996. **270**(4 Pt 1): p. E627-33.

103. Fern, E.B., P.J. Garlick, and J.C. Waterlow, *Apparent compartmentation of body nitrogen in one human subject: its consequences in measuring the rate of whole-body protein synthesis with 15N.* Clin Sci (Lond), 1985. **68**(3): p. 271-82.

104. Stein, T.P., et al., *Effect of reduced dietary intake on energy expenditure, protein turnover, and glucose cycling in man.* Metabolism, 1991. **40**(5): p. 478-83.

105. Haqq, A.M., et al., *The Study of the Effects of Diet on Metabolism and Nutrition (STEDMAN) weight loss project: Rationale and design.* Contemp Clin Trials, 2005. **26**(6): p. 616-25.

106. Lien, L.F., et al., *The STEDMAN project: biophysical, biochemical and metabolic effects of a behavioral weight loss intervention during weight loss, maintenance, and regain.* OMICS, 2009. **13**(1): p. 21-35.

107. Huffman, K.M., et al., *Exercise-induced changes in metabolic intermediates, hormones, and inflammatory markers associated with improvements in insulin sensitivity.* Diabetes Care, 2011. **34**(1): p. 174-6.

108. Medicine, A.C.o.S., *ACSM's guidelines for exercise testing and prescription*. 2013: Lippincott Williams & Wilkins.

109. Orr, G.W., et al., *A computer linear regression model to determine ventilatory anaerobic threshold.* Journal of Applied Physiology, 1982. **52**(5): p. 1349-1352.

110. Moshfegh, A.J., et al., *The US Department of Agriculture Automated Multiple-Pass Method reduces bias in the collection of energy intakes*–. The American journal of clinical nutrition, 2008. **88**(2): p. 324-332.

111. Beck, T.W., *The importance of a priori sample size estimation in strength and conditioning research.* J Strength Cond Res, 2013. **27**(8): p. 2323-37.

112. DeFronzo, R.A. and D. Tripathy, *Skeletal muscle insulin resistance is the primary defect in type 2 diabetes.* Diabetes Care, 2009. **32**(suppl 2): p. S157-S163.

113. Turk, Y., et al., *High intensity training in obesity: a Meta-analysis.* Obes Sci Pract, 2017. **3**(3): p. 258-271.

114. Heydari, M., J. Freund, and S.H. Boutcher, *The effect of high-intensity intermittent exercise on body composition of overweight young males.* Journal of obesity, 2012. **2012**: p. 480467.

115. Viana, R.B., et al., *Is interval training the magic bullet for fat loss? A systematic review and meta-analysis comparing moderate-intensity continuous training with high-intensity interval training (HIIT).* British journal of sports medicine, 2019. **53**(10): p. 655-664.

116. Borsheim, E., et al., *Essential amino acids and muscle protein recovery from resistance exercise.* American journal of physiology. Endocrinology and metabolism, 2002. **283**(4): p. E648-57.

117. Paddon-Jones, D., et al., *Exogenous amino acids stimulate human muscle anabolism without interfering with the response to mixed meal ingestion.* American journal of physiology. Endocrinology and metabolism, 2005. **288**(4): p. E761-7.

118. Smith-Ryan, A.E., *Enjoyment of high-intensity interval training in an overweight/obese cohort: a short report.* Clin Physiol Funct Imaging, 2017. **37**(1): p. 89-93.

119. Maillard, F., B. Pereira, and N. Boisseau, *Effect of high-intensity interval training on total, abdominal and visceral fat mass: a meta-analysis.* Sports medicine, 2018. **48**(2): p. 269-288.

120. Hirsch, K.R., et al., *Visceral adipose tissue normative values in adults from the United States using GE Lunar iDXA.* Clin Physiol Funct Imaging, 2019. **39**(6): p. 407-414.

121. Phillips, S.M., S. Chevalier, and H.J. Leidy, *Protein "requirements" beyond the RDA: implications for optimizing health.* Applied Physiology, Nutrition, and Metabolism, 2016. **41**(5): p. 565-572.

122. Abe, T., et al., *Influence of adipose tissue mass on DXA-derived lean soft tissue mass in middle-aged and older women.* Age, 2015. **37**(1): p. 2.

123. Loenneke, J., P. Loprinzi, and T. Abe, *The prevalence of sarcopenia before and after correction for DXA-derived fat-free adipose tissue.* European journal of clinical nutrition, 2016. **70**(12): p. 1458-1460.

124. Kumar, V., et al., *Human muscle protein synthesis and breakdown during and after exercise.* Journal of applied physiology, 2009. **106**(6): p. 2026-2039.

125. Perry, C.G., et al., *Repeated transient mRNA bursts precede increases in transcriptional and mitochondrial proteins during training in human skeletal muscle.* The Journal of physiology, 2010. **588**(23): p. 4795-4810.

126. Rolfe, D. and G.C. Brown, *Cellular energy utilization and molecular origin of standard metabolic rate in mammals.* Physiological reviews, 1997. **77**(3): p. 731-758.

127. Paddon-Jones, D., et al., *Exogenous amino acids stimulate human muscle anabolism without interfering with the response to mixed meal ingestion.* American Journal of Physiology-Endocrinology and Metabolism, 2005. **288**(4): p. E761-E767.

128. Bohe, J., et al., *Human muscle protein synthesis is modulated by extracellular, not intramuscular amino acid availability: a dose-response study.* J Physiol, 2003. **552**(Pt 1): p. 315-24.

129. Forbes, S.C. and G. Bell, *Whey protein isolate supplementation while endurance training does not alter cycling performance or immune responses at rest or after exercise.* Frontiers in nutrition, 2019. **6**: p. 19.

130. Knuiman, P., et al., *Protein supplementation elicits greater gains in maximal oxygen uptake capacity and stimulates lean mass accretion during prolonged endurance training: a double-blind randomized controlled trial.* The American journal of clinical nutrition, 2019. **110**(2): p. 508-518.

131. Smith, A.E., et al., *Effects of β-alanine supplementation and high-intensity interval training on endurance performance and body composition in men; a double-blind trial.* Journal of the International Society of Sports Nutrition, 2009. **6**(1): p. 5.

132. Hoffmann, J.J., et al., *Specificity effects of run versus cycle training on ventilatory threshold.* European journal of applied physiology and occupational physiology, 1993. **67**(1): p. 43-47.

133. Batacan, R.B., et al., *Effects of high-intensity interval training on cardiometabolic health: a systematic review and meta-analysis of intervention studies.* British journal of sports medicine, 2017. **51**(6): p. 494-503.

134. Little, J.P., et al., *Low-volume high-intensity interval training reduces hyperglycemia and increases muscle mitochondrial capacity in patients with type 2 diabetes.* Journal of applied physiology, 2011. **111**(6): p. 1554-1560.

135. Huffman, K.M., et al., *Relationships between circulating metabolic intermediates and insulin action in overweight to obese, inactive men and women.* Diabetes Care, 2009. **32**(9): p. 1678-83.

136. Newgard, C.B., et al., *A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance.* Cell Metab, 2009. **9**(4): p. 311-26.

137. Kim, I.-Y., et al., *Short term elevation in dietary protein intake does not worsen insulin resistance or lipids in older adults with metabolic syndrome: a randomized-controlled trial.* BMC nutrition, 2017. **3**(1): p. 33.

138. Bussau, V.A., et al., *The 10-s maximal sprint: a novel approach to counter an exercise-mediated fall in glycemia in individuals with type 1 diabetes.* Diabetes Care, 2006. **29**(3): p. 601-606.

139. Gaitanos, G.C., et al., *Human muscle metabolism during intermittent maximal exercise.* Journal of applied physiology, 1993. **75**(2): p. 712-719.

140. Vincent, S., et al., *Plasma glucose, insulin and catecholamine responses to a Wingate test in physically active women and men.* European journal of applied physiology, 2004. **91**(1): p. 15-21.

141. Kessler, H.S., S.B. Sisson, and K.R. Short, *The potential for high-intensity interval training to reduce cardiometabolic disease risk.* Sports medicine, 2012. **42**(6): p. 489-509.

142. Sokoloff, N.C., M. Misra, and K.E. Ackerman, *Exercise, training, and the hypothalamic-pituitary-gonadal axis in men and women*, in *Sports Endocrinology*. 2016, Karger Publishers. p. 27-43.

143. Kim, I.-Y., et al., *Consumption of a Specially-Formulated Mixture of Essential Amino Acids Promotes Gain in Whole-Body Protein to a Greater Extent than a Complete Meal Replacement in Older Women with Heart Failure.* Nutrients, 2019. **11**(6): p. 1360.

144. Forbes, S.C., et al., *Supplements and Nutritional Interventions to Augment High-Intensity Interval Training Physiological and Performance Adaptations—A Narrative Review.* Nutrients, 2020. **12**(2): p. 390.

145. Kephart, W.C., et al., *Ten weeks of branched-chain amino acid supplementation improves select performance and immunological variables in trained cyclists.* Amino acids, 2016. **48**(3): p. 779-789.

146. Janssen, I., et al., *Skeletal muscle mass and distribution in 468 men and women aged 18-88 yr.* Journal of applied physiology, 2000. **89**(1): p. 81-8.

147. Fothergill, E., et al., *Persistent metabolic adaptation 6 years after "The Biggest Loser" competition.* Obesity (Silver Spring), 2016. **24**(8): p. 1612-9.

148. Goodpaster, B.H., et al., *The loss of skeletal muscle strength, mass, and quality in older adults: the health, aging and body composition study.* The Journals of Gerontology Series A: Biological Sciences and Medical Sciences, 2006. **61**(10): p. 1059-1064.

149. Hirsch, K.R., et al., *Influence of segmental body composition and adiposity hormones on resting metabolic rate and substrate utilization in overweight and obese adults.* Journal of endocrinological investigation, 2017. **40**(6): p. 635-643.

150. Corcoran, M.P., S. Lamon-Fava, and R.A. Fielding, *Skeletal muscle lipid deposition and insulin resistance: effect of dietary fatty acids and exercise.* The American journal of clinical nutrition, 2007. **85**(3): p. 662-77.

151. Ahtiainen, J.P., et al., *Panoramic ultrasonography is a valid method to measure changes in skeletal muscle cross-sectional area.* European journal of applied physiology, 2010. **108**(2): p. 273.

152. Narici, M., M. Franchi, and C. Maganaris, *Muscle structural assembly and functional consequences.* Journal of Experimental Biology, 2016. **219**(2): p. 276-284.

153. Tipton, K.D., et al., *Timing of amino acid-carbohydrate ingestion alters anabolic response of muscle to resistance exercise.* Am J Physiol Endocrinol Metab, 2001. **281**(2): p. E197-206.

154. Kim, I.Y., et al., *Applications of stable, nonradioactive isotope tracers in in vivo human metabolic research.* Exp Mol Med, 2016. **48**: p. e203.

155. Mangine, G.T., et al., *Resistance training does not induce uniform adaptations to quadriceps.* PLoS One, 2018. **13**(8).

156. Haun, C.T., et al., *Muscle fiber hypertrophy in response to 6 weeks of high-volume resistance training in trained young men is largely attributed to sarcoplasmic hypertrophy.* PLoS One, 2019. **14**(6).

157. Tarnopolsky, M.A., *Sex differences in exercise metabolism and the role of 17-beta estradiol.* Med Sci Sports Exerc, 2008. **40**(4): p. 648-54.

158. Imboden, M.T., et al., *Cardiorespiratory fitness and mortality in healthy men and women.* Journal of the American college of cardiology, 2018. **72**(19): p. 2283-2292.